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Poster Session I

M2. Blockade of Kappa Opioid Receptors Reduces Footshock Effects on Startle

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Background: Brain kappa-opioid receptors (KORs) have been implicated in the behavioral consequences of stress, such as drug seeking and depressive-like behavior. Previously, we have shown that systemic KOR antagonism produces anxiolytic-like effects in tests of both conditioned and unconditioned fear. The present studies were designed to further characterize interactions between KOR systems and stress-induced behavior using footshock as a stressor, the effects of which can be measured by potentiation of the acoustic startle reflex. This potentiation was investigated after systemic blockade or constitutive deletion of KORs. Recently, it has been shown that stress induces KOR phosphorylation in the nucleus accumbens, where KORs are expressed on the terminals of dopamine (DA) neurons originating in the ventral tegmental area (VTA). To characterize the involvement of this specific population of KORs we generated a conditional knockout in which KORs are selectively deleted in DA-containing neurons.
Methods: To study the effects of KOR on stress-induced behaviors, mice were designed with loxP sites flanking exon 3 of the KOR gene. This arrangement enables gene inactivation by Cre recombinase and generation of our two KOR KO lines. Floxed mice were crossed to either a ubiquitous Cre line (EIIaCre) to generate constitutive KOR KOs or DATCre to generate conditional KOs lacking KORs specifically in DA transporter (DAT)-expressing cells, such as those found in the VTA and substantia nigra (SN). Knockout of the receptor was confirmed with autoradiography using a radiolabeled KOR agonist ([H^3]U-69593). Further, mRNA analyses were performed on brain tissue samples using qPCR to demonstrate specificity of deletion. To test the effect of KOR antagonism on shock-potentiated startle, mice were matched into groups with equivalent baseline startle and given an intraperitoneal injection of the KOR antagonist JDTic or vehicle 24 hr prior to testing to accommodate the slow onset and persistent actions of this drug. In the test session, mice received a baseline startle test followed by ascending footshock amplitudes (0.2 mA, 0.4 mA, and 0.8 mA) each followed by a startle test. The following day, mice were given a final startle test. KOR KO mice were subjected to the same behavioral protocol without administration of JDTic. Data were analyzed using appropriate ANOVAs and significant effects were analyzed using *post hoc* Bonferroni tests. Experiments were conducted in accordance with National Institutes of Health and McLean Hospital guidelines for the care and use of laboratory animals.
Results: Autoradiography demonstrated a lack of KOR binding in constitutive KOR KO mice and reduced binding in conditional DATCre KOs. Further, qPCR demonstrated no detectable KOR mRNA in KOs and reduced KOR mRNA specifically in the VTA/SN of DATCre KOs. Blockade of KOR receptors by JDTic attenuated footshock induced increases in startle. When shocked mice were returned to the testing chamber on the following day, JDTic pretreated mice continued to show significantly decreased context conditioning. In comparison, constitutive KOR KO mice had similar levels of footshock-potentiated startle compared to littermate controls. Like JDTic treated mice, DATCre KOs showed significantly lower levels of potentiation than controls.
Conclusions: These studies used two novel lines of KOR KO mice, as demonstrated by reduced levels of KOR mRNA and KOR binding, to follow up initial findings that systemic blockade of KORs with JDTic reduces startle potentiation after footshock. Interestingly, constitutive KOR KO mice show equivalent levels of footshock-potentiated startle compared to wild type littermates, whereas KOR deletion restricted to DAT expressing neurons (DATCre KO) was sufficient to reduce potentiated startle following the first two blocks of footshock. This pattern of results suggests that compensatory adaptations occurring outside brain DA systems may offset behavioral changes that are caused by KOR deletion within brain DA systems in constitutive KOR KOs. Overall, these data implicate KORs of midbrain dopaminergic cells in the manifestation of stress, and provide additional evidence that disruption of KOR function reduces stress-induced anxiety-like behavior. JDTic treated mice had acute blockade of KORs, while KOR KO mice had a lifelong absence of KORs. Thus, the finding that systemic KOR antagonism produces anti-stress effects that are not seen in the constitutive KOR KO mice suggests the importance of neuroadaptations occurring during brain development.
Keywords: kappa opioid, stress, startle, mouse
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M3. Imparied Mesolimbic Regulation of Prefrontal Glutamate and Acetylcholine Release Accompanies Cognitive Inflexibility in Two Animal Models of Schizophrenia

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Background: Executive control, under conditions of enhanced cognitive demands, is mediated by top-down recruitment, by the prefrontal cortex (PFC), of a network that includes the ventral hippocampus (VH), nucleus accumbens (NAC) and basal forebrain. Several of the cognitive deficits seen in schizophrenia (SZ), including, attention and cognitive flexibility, are believed to emerge from impaired prefrontal cholinergic and glutamatergic transmission arising from dysregulations in these cortical-subcortical interactions.
Methods: Neurochemical and cognitive deficits were compared in two neurodevelopmental rat models of SZ: 1) elevated levels of the endogenous alpha7 nAChR antagonist kynurenic acid (KYNA) following daily exposure of the dam/litter (GD 14/15-PD21) to mash containing the precursor kynurenine and 2) transient inactivation of impulse flow in VH following bilateral local infusions of TTX during a sensitive period of development (PD7). Testing was conducted in young adults (PD56-80). Mesolimbic stimulation was produced with infusions of NMDA (0.05 - 0.3 μg/0.4 μL) into the NAC shell. The mesolimbic regulation of prefrontal glutamate release was measured by a glutamate-sensitive microelectrode array and ACh levels using microdialysis. As a measure of cognitive flexibility, performance in an attentional set-shifting task (ID/ED digging task) was determined, in separate groups of

animals, with and without acute pretreatment with an alpha 7 n AChR agonist [galantamine or SSR18711 (both at 3.0 mg/kg, i.p.)].
Results: Rats exposed to kynurenine during development exhibited elevated levels of forebrain KYNA on PD2, PD21, and even as adults (long after exposure had stopped). In adult controls, intra-NAC NMDA produced a dose-dependent phasic increase in prefrontal glutamate release as well as a tonic increase in extracellular ACh levels. NMDA-induced cortical glutamate and ACh release was almost eliminated in rats that had developed with elevated KYNA levels. In addition, these rats exhibited selective impairments [reversal (REV) and extra-dimensional shift (EDS)] in the set-shifting task which were normalized by pretreatment with galantamine. Similar results were seen following transient postnatal inactivation of the VH with TTX. As adults, these rats failed to display the NMDA-induced increases in prefrontal glutamate or ACh. They also exhibited deficits in REV and EDS stages of the behavioral task that were normalized following pretreatment with SSR18711.
Conclusions: The striking convergence in the neurochemical and cognitive deficits seen in these two different animal models highlights the multiple etiological pathways in SZ. Anomalies that interfere with the functional connectivity and transmitter release within this hippocampal-mesolimbic-prefrontal network, during a sensitive period of development, are likely to yield a common maturational profile of cognitive impairments that resembles elements of those seen in SZ.
Keywords: cognition; glutamate; acetylcholine; cortex; schizophrenia
Disclosure: M. Pershing, Nothing to Disclose; D. Bortz, Nothing to Disclose; A. Pocivavsek, Nothing to Disclose; M. Sarter, Nothing to Disclose; R. Schwarcz, **Part 4:** Research support from Mitsubishi and BMS.; J. Bruno, Nothing to Disclose.

M4. Transition to Dorsolateral Striatal Dopamine Control of Cocaine Seeking Behavior is Predicted by Trait Impulsivity

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Background: Addiction has been suggested to be characterized by a loss of control over drug seeking, possibly due to the development of maladaptive drug-seeking habits that emerge concurrently with recruitment of dorsolateral striatal (DLS) dopamine control over seeking behavior. The involvement of DLS dopamine signaling has been shown gradually to emerge as cocaine seeking undergoes the transition from goal-directed to habitual, being elicited and maintained by response-contingent presentations of drug associated conditioned stimuli (CSs) acting as conditioned reinforces. Factors that influence the rate of this transition from goal-directed actions to habits have yet to be elucidated. Trait impulsivity has been found to be predictive of several measures of addictive behavior including escalation of cocaine intake, compulsivity (resistance to punished cocaine self-administration), and the propensity to relapse after punishment-induced abstinence. The present study investigated whether individual differences in impulsivity, defined by the number of premature responses in the five-choice serial reaction time task (5-CSRTT) – 'waiting' impulsivity, predicted the propensity for drug seeking to be devolved to DLS control.
Methods: Rats were trained on the 5-CSRTT then screened for the number of premature responses on probe long intertrial-interval sessions to identify trait low impulsive (LI; <20 premature responses) and high impulsive (HI; >50 premature responses) individuals. HI and LI groups were then trained to self-administer cocaine (0.25 mg/infusion) under a FR1 schedule with infusions occurring in the presence of a 20-sec light CS. Following 5 training sessions, dopamine transmission was blocked in the DLS via bilateral infusions of the D1/D2 dopamine receptor antagonist α-flupenthixol (0, 5, 10, or 15 μg/side) immediately prior to 15-min cocaine seeking test sessions in which each lever press was only reinforced by a 1-sec presentation of the CS [FI15(FR1:S)]. The response requirement was then gradually increased across sessions to a FR10(FR4:S) second-order schedule in which every forth lever press resulted in a 1-sec CS presentation; cocaine and the 20-sec CS were presented on the tenth FR4 completion. Following 5 training sessions on this schedule, α-flupenthixol was again infused into the DLS during 15-min cocaine seeking tests in which every forth lever press was only reinforced by the 1-sec CS presentation [FI15(FR4:S)]. The response requirement was then increased to a FI15(FR10:S) second-order schedule in which cocaine seeking was maintained over 15-min delays by 1-sec CS presentations on every tenth lever press. Following 15 sessions of training on this schedule, cocaine seeking tests with intra-DLS dopamine receptor blockade were again conducted.
Results: When HI and LI groups were analyzed together, there was no effect of DLS dopamine receptor blockade in the early-stage tests, when training had been under an FR1 schedule of reinforcement. By the transition-stage tests, when training had been under the FR10(FR4:S) schedule of reinforcement, 10 and 15 μg/side α-flupenthixol reduced cocaine-seeking relative to vehicle. At the final, well-established stage of testing, following extended training under the FI15(FR10:S) schedule of reinforcement, all three α-flupenthixol doses reduced cocaine seeking. However, analyzing the effects of intra-DLS α-flupenthixol on LI and HI groups separately revealed differences in the point of transition to DLS control of cocaine seeking. The LI group showed the same pattern as the full cohort: no effect of DLS dopamine receptor blockade in the early-stage test, and in the transition-stage and well-established stage tests, a significant dose-dependent reduction in cocaine seeking. However, HI rats displayed a slower emergence of DLS dopamine control over cocaine seeking. There was only an effect of intra-DLS α-flupenthixol infusions by the final test in the well-established stage.
Conclusions: Combined, these results indicate that trait impulsivity affects the recruitment of dopaminergic circuitry at early and well-established stages of performance as cocaine seeking undergoes a transition from goal-directed to habitual and under the control of contingent presentations of a cocaine-associated conditioned reinforcer. High impulsivity predicted a slowed recruitment of DLS dopaminergic circuitry to control cocaine-seeking behavior. When considered alongside other measures of addictive behavior including escalated intake, lower sensitivity to punished self-administration, and greater relapse following abstinence, these results suggest that cocaine seeking persists as goal-directed in HI rats, a persistence that is perhaps related to differences in ventral striatal dopaminergic transmission that are associated with the impulsive trait.
Keywords: impulsivity cocaine habit dorsal striatum individual differences
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M5. Basolateral and Central Nuclei of the Amygdala Required for the Transition to Dorsolateral Dopamine Control over Habitual Cocaine Seeking

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Background: A primary feature of addictive behavior is the progression from casual drug taking to drug seeking as a compulsive habit. The transition from goal-directed to habitual drug seeking behavior arises through a progressive recruitment of dopamine circuitry that gradually shifts control over responding

from more ventral and medial to more dorsal and lateral regions of the striatum. Thus, habitual drug seeking under a second-order schedule of reinforcement, in which high response levels are maintained over long intervals through contingent presentations of a drug-paired conditioned stimulus (CS), depends upon dorsolateral striatal (DLS) dopamine transmission that is recruited by the core of the nucleus accumbens (NAcC). This habitual drug seeking is elicited and maintained by CSs that acquire their conditioned reinforcing properties (or incentive value) by associative processing in the basolateral amygdala (BLA). Since the BLA projects to the NAcC and not the DLS, we hypothesized that it is through this projection, and subsequent activation of serial connections via midbrain dopamine neurons to influence the DLS, that DLS-dependent, habitual drug seeking is engaged by associative control exerted by the amygdala. However, the BLA also projects to the central nucleus of the amygdala (CeN), which also projects to midbrain dopamine neurons, especially the substantia nigra, providing an alternative route to influence DLS processing. The present study investigated these two possible routes by which the amygdala may relay incentive information to the striatum using a functional disconnection procedure and testing cue-controlled cocaine seeking at early, mid, and late, well-established, stages in the performance of cocaine-seeking behavior.
Methods: Rats were assigned to one of three groups: DLS-DLS Control; BLA-DLS; CeN-DLS. The BLA-DLS and CeN-DLS groups received unilateral lesions of the BLA or CeN, respectively; DLS-DLS Control rats were given sham lesions. All rats were then implanted with bilateral cannulae targeting the DLS. Groups were then trained to self-administer cocaine (0.25 mg/infusion) under a FR1 schedule with infusions occurring in the presence of a 20-sec light CS. Following acquisition, dopamine transmission was blocked in the DLS via intracranial infusions (bilateral for DLS-DLS Control Group; unilateral, contralateral to the lesion in BLA-DLS and CeN-DLS groups) of the D1/D2 dopamine receptor antagonist α-flupenthixol (0 and 10 μg/side) immediately prior to 15-min cocaine seeking test sessions in which each lever press was only reinforced by a 1-sec presentation of the CS [FI15(FR1:S)]. The response requirement was then gradually increased across sessions to a FR10(FR4:S) second-order schedule in which every forth lever press resulted in a 1-sec CS presentation; cocaine and the 20-sec CS were presented on the tenth FR4 completion. α-Flupenthixol was again infused into the DLS during 15-min cocaine seeking tests in which every forth lever press was only reinforced by the 1-sec CS presentation [FI15(FR4:S)]. The response requirement was further increased to a FI15(FR10:S) second-order schedule in which cocaine seeking was maintained over 15-min delays by 1-sec CS presentations on every tenth lever press. Cocaine seeking tests with intra-DLS dopamine receptor blockade (0, 5, 10, and 15 μg/side) were again conducted. In the final set of tests, α-flupenthixol (0 and 10 μg) was infused unilaterally in the DLS-DLS Control group and unilaterally, ipsilaterally in the BLA-DLS and CeN-DLS groups.
Results: Functional disconnections using a unilateral lesion of either region of the amygdala combined with contralateral dopamine receptor blockade in the DLS, disrupted performance to a degree that was similar to the effect of bilateral DLS dopamine receptor blockade. At the early-stage tests in all groups, cocaine seeking was unaffected either by bilateral α-flupenthixol infusions into the DLS (DLS-DLS Control group) or by α-flupenthixol infusions contralateral to lesions in the BLA-DLS or CeN-DLS groups. At the mid-stage tests when training had been under an FR10(FR4:S) schedule of reinforcement, bilateral infusions of 10 μg α-flupenthixol reduced lever pressing in the DLS-DLS Control group. However, the BLA-DLS and CeN-DLS disconnections did not at this stage affect cocaine seeking. In the well-established stage tests following extended training under the FI15(FR10:S) schedule of reinforcement, α-flupenthixol infusions dose-dependently decreased cocaine seeking in all groups. Unilateral infusions of α-flupenthixol alone, or when made ipsilateral to BLA or CeN lesions, had no effect on cocaine seeking, highlighting the functional impact of the disconnection.
Conclusions: These results demonstrate that disconnection of either the BLA or the CeN from the DLS disrupted the recruitment of dopaminergic circuitry as cocaine seeking behavior becomes progressively more habitual. Therefore, it is possible that the incentive value assigned to the CS in the BLA is routed via the CeN to influence the DLS control over habitual cocaine seeking following extensive training.
Keywords: cocaine habit amygdala dorsal striatum nucleus accumbens core
Disclosure: J. Murray, Nothing to Disclose; D. Belin, Nothing to Disclose; B. Everitt, Nothing to Disclose.

M6. GAD67 Downregulation in Specific Interneuronal Subpopulations Leads to Distinct Behavioral Phenotypes

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Background: Glutamic acid decarboxylase 67 kDa (GAD67, encoded by the GAD1 gene) is the primary GABA-producing enzyme in the brain and is a main phenotypic marker of interneurons. Interneurons can be further classified by their expression of molecular markers such as neuropeptide Y (NPY), cholecystokinin (CCK), somatostatin (SOM), or parvalbumin (PV). The different interneuronal subclasses provide various types of inhibition to the glutamatergic projection neurons. PV-, SOM-, CCK- and NPY-expressing interneurons appear to have altered molecular content in multiple psychiatric disorders, however, the ability of each these interneuronal sub-classes to regulate behavior has not been fully explored to date.
Methods: We hypothesized that *in vivo* GAD67 downregulation across various subpopulations of interneurons will lead to robust, distinct and reproducible behavioral alterations in transgenic mice. To test this, we developed separate lines of bacterial artificial chromosome (BAC) driven, GAD-1 silenced transgenic mice that exclusively suppressed GAD67 protein expression in the NPY + and CCK + interneuronal subpopulations. BAC constructs, containing the NPY or CCK promoter-enhancer elements, were modified to generate an eGFP reporter and a synthetic microRNA (miRNA) targeted against GAD1 mRNA. This manipulation achieved suppressed GAD67 expression in NPY + or CCK + interneuron subclasses, with an ability to easily visualize the affected cells. Construct efficacy and cell-type expression specificity were assessed using double-label immunohistochemistry. NPY-BAC/Gad1-miRNA and CCK-BAC/Gad1-miRNA transgenic mice ($n = 12$ per group) and their wild-type littermates ($n = 12$ per group) were subjected to a broad behavioral testing battery, including Irwin Screen, learning, memory, anxiety/risk-taking, social behavior, sensorimotor gating, locomotor activity, and amphetamine sensitivity (3 mg/kg) assessments.
Results: NPY-BAC/Gad1-miRNA and CCK-BAC/Gad1-miRNA constructs effectively suppressed GAD67 expression in their targeted interneuronal subpopulations: while eGFP was readily detected in NPY + cells and CCK + cells, respectively, GAD67 was undetectable in the construct-expressing cells. In comparison to their matched control littermates, there were no differences in general neurological/neuromuscular functions in either line. However, the two transgenic mice lines displayed distinct behavioral phenotypes: CCK-BAC/Gad1-miRNA transgenic mice were hypoactive across several different testing paradigms, while the NPY-BAC/Gad1-miRNA transgenic mice displayed a "less-anxious" or "less risk-averse" phenotype in both elevated zero maze and light-dark exploration. Furthermore, the NPY-BAC/Gad1-miRNA transgenic mice showed a dramatically increased sensitive to amphetamine compared to littermate controls (621% increase, $p < 0.001$).

Conclusions: Our experiments lead us to two main conclusions. First, downregulation of GAD67 across different interneuronal subpopulations is sufficient to alter behavior and second, the resulting behavioral phenotypes appear to be cell-type specific. We also observed that many of these behavioral changes appear to be associated with dopaminergic system dysfunction, suggesting that altering GABA system leads to a profound dopaminergic dysregulation and this warrants further examination. Ongoing research will explore effects of GAD67 downregulation in additional interneuron populations using additional mouse lines recently created in our laboratory.
Keywords: Transgenic, GABA, GAD1, schizophrenia, animal model, behavior, dopamine
Disclosure: M. Schmidt, Nothing to Disclose; K. Garbett, Nothing to Disclose; S. Horvath, Nothing to Disclose; K. Mirnics, Nothing to Disclose.

M7. Brief Repeated Cortico-striatal Hyperstimulation Generates Chronic OCD-relevant Behavior

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Background: Obsessive Compulsive Disorder (OCD) is a chronic, severe mental illness that affects 2-3% of people worldwide. Despite the severity and prevalence of OCD, the pathophysiology remains unclear, limiting development of new treatments. Multiple lines of evidence suggest that dysregulation within cortico-striato-thalamo-cortical (CSTC) circuits is correlated with OCD. Functional imaging studies in OCD patients demonstrate increased activity in CSTC circuits after symptom provocation; conversely, successful / psychotherapy is associated with reduced OFC and caudate activity. This leads to the hypothesis that over activation of OFC-striatal projections underlies OCD symptoms; however, this question cannot be definitively tested in humans. It has therefore not been possible to directly test whether and how abnormalities in CSTC circuit function lead to OCD-relevant behaviors.
Methods: In this study, we have directly tested whether hyperactivation of OFC-striatal projections leads to OCD-relevant behaviors in mice. To do so, we used optogenetic technology to simulate CSTC hyperactivation observed in OCD patients.
Viral injections: We generated mice that allowed us to specifically stimulate OFC-VMS projections by using a Cre-inducible adenoviral vector carrying the gene encoding channelrhodopin (ChR2) fused to YFP [pAAV-Ef1a-DIO-ChR2 (H134R)-EYFP]. Virus was stereo tactically injected into OFC of EMX-Cretransgenic mice. Fiberoptics were simultaneously implanted into ventromedial striatum. Cre expression in cortex led to sustained cortical-specific expression of ChR2-EYFP. Two weeks post-injection, EYFP staining was seen in OFC cell bodies and axons projecting to VMS patches, demonstrating targeting of OFC-VMS projections. Controls received sham injections using a virus expressing YFP alone. *Behavioral testing:* Stimulation was performed daily for 5 days; each day, open field and grooming tests were performed. Mice were stimulated in the open field with a 473 nm laser using 10msec pulses at 10 Hz for 5 minutes. We also observed behavior for 5 minutes before and after stimulation to observe 1) acute behavioral impact of the stimulus, and 2) inter-stimulus effects on behavior. In addition, grooming was assessed 1 h post-stimulation. Standard behavioral output including locomotor and stereotypic measures was obtained. Videotapes were hand scored to determine number, duration, and quality of grooming episodes. Data were analyzed both within and between subjects using repeated-measures ANOVA, with laser-stimulation as the within subjects factor. *Histological examination/ verification of targeting*: Brains were extracted after perfusion following the last behavioral test. Correct targeting of viral injection and fiber optic implantation was verified. Confocal microscopy was used to examine OFC and striatum for gross anatomical abnormalities and changes in cellular morphology resulting from stimulation. ChR2-expressing cells were identified by immune staining for YFP. *Electrophysiological verification*: Striatal extracellular recordings were performed both *in vitro* (using cortico-striatal slices), and *in vivo* (recording alongside the fiber optic cannulae).
Results: We found that brief repeated hyperactivation of OFC-ventromedial striatum (VMS) projections led to repetitive grooming, a mouse behavior linked to OCD. Increased grooming persisted for 2 weeks after cessation of repeated stimulation, and was reversed by chronic fluoxetine treatment. In contrast, *acute* hyperactivation had no effect on grooming behavior. This effect was spatially specific, since chronic stimulation of infralimbic/ prelimbic (IL/PL) cortex-VMS projections did not alter grooming behavior. Perseverative grooming was correlated with decreased OFC-VMS theta power.
Conclusions: We have provided the first evidence that hyperactivation of specific cortico-striatal projections directly generates OCD-relevant behaviors. Furthermore, we have shown that repetitive grooming, once established, persists in the absence of further direct circuit hyperactivation, but is resolved using a treatment regimen effective in a subset of OCD patients. Finally, we have found an electrophysiologic signature that correlates with the observed behavioral change. Our approach may provide a general template for modeling circuit-specific disease states using combinatorial optogenetic methodology. This is relevant for many complex developmental neuropsychiatric disorders other than OCD, including schizophrenia and autism.
Keywords: Obsessive Compulsive Disorder (OCD), optogenetics, grooming, mouse model, fluoxetine
Disclosure: S. Ahmari, Nothing to Disclose.

M8. Deficiency of Schnurri-2, an MHC Enhancer Binding Protein, Induces Mild Chronic Inflammation in the Brain and Confers Molecular, Neuronal, and Behavioral Phenotypes Related to Schizophrenia

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Background: Schnurri-2 (Shn-2), an NF-kappa B site-binding protein, tightly binds to the enhancers of major histocompatibility complex (MHC) class I genes and inflammatory cytokines, which have been shown to harbor common variant single nucleotide polymorphisms associated with schizophrenia. Although genes related to immunity are implicated in schizophrenia, there has been no study showing that their mutation or knockout results in schizophrenia.
Methods: Behavioral, molecular, and physiological phenotyping of the Shn-2 KO mice were conducted. Also, we tried to rescue the phenotypes by anti-psychotics and immunosuppressants.
Results: Shn-2 knockout mice have behavioral abnormalities that strongly resemble those of schizophrenics. The mutant brain demonstrated numerous schizophrenia-related phenotypes, including transcriptome/proteome changes remarkably similar to those of postmortem schizophrenia patients, decreased parvalbumin and GAD67 levels, increased theta power on electroencephalograms, and a thinner cortex. Dentate gyrus granule cells failed to mature in mutants, a previously proposed endophenotype of schizophrenia. Shn-2 knockout mice also exhibited mild chronic inflammation of the brain, as evidenced by increased inflammation markers, such as GFAP and NADH/NADPH oxidase p22 phox, and their genome-wide gene expression pattern similar to various inflammatory conditions. Chronic administration of

anti-inflammatory drugs reduced the expression of GFAP in the hippocampus, and reversed the deficits of working memory and nest building behaviors in the Shn-2 KO mice.
Conclusions: Shn-2 knockout mice have an outstanding face, construct and predictive validity as a genetic animal model of schizophrenia. These results suggest that genetically-induced changes in immune system could be a predisposing factor in schizophrenia.
Keywords: NF-kB, immature dentate gyrus (iDG), MHC, inflammation, astrocyte
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M9. Serine Racemase Knockout Mice, a Genetic Model of NMDA Receptor Hypofunction, Exhibit Impaired Hippocampal Neuroplasticity That Can be Rescued by D-serine Treatment

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Background: There is substantial evidence that hypofunction of the *N*-methyl-D-aspartate receptor (NMDAR) is a core pathophysiological mechanism underlying schizophrenia. In addition, there are abnormalities in hippocampal structure and function in schizophrenia. We have previously demonstrated that serine racemase knockout (SR−/−) mice exhibit reductions in the spine density of dentate granule cells, brain derived neurotrophic factor (BDNF), and microRNA (miR)-132, all of which are consistent with what is observed in schizophrenia.
Methods: Baseline synaptic transmission was assayed with input-output curves and paired pulse ratio at the perforant path (PP) to dentate gyrus (DG) synapses. To examine the role of endogenous D-serine in the mechanisms of NMDA-dependent synaptic plasticity, we stimulated the PP and recorded the field excitatory postsynaptic potentials (fEPSPs) in the DG of the hippocampus in slices from wild-type (WT) and SR−/− mice. Western blot and enzyme-linked immunosorbent assays (ELISA) were used to measure protein changes. For chronic D-serine treatment, WT and SR−/− mice were given once daily subcutaneous injections of either vehicle (0.9% saline) or D-serine (150 mg/kg) for 21 days and were sacrificed 24 hours after the last injection for subsequent analyses. D-serine tissue and serum content were measured using high-performance liquid chromatography. Renal glomeruli were examined in haematoxylin-eosin stained kidney sections to assess the potential nephrotoxicity of D-serine.
Results: Although baseline synaptic transmission remained unchanged in slices from SR−/− mice, the magnitude of long-term potentiation (LTP) induced by a 1-s train of 100 Hz stimulation was significantly reduced in SR−/− mice. Since we previously demonstrated that SR−/− mice have reduced BDNF expression and TrkB receptor activation in the hippocampus, we examined the activity of signaling pathways downstream of TrkB. We found that although the total amount of Akt protein did not differ between genotypes, the amount of phosphorylated Akt (p-Akt; active state) was reduced in SR−/− mice. In addition, the amount of p-mTOR (Ser2448; mammalian target of rapamycin), which is phosphorylated by Akt, was reduced in SR−/− mice. The reduced levels of p-Akt and p-mTOR were also found in the synaptoneurosome fraction. We then determined whether these neuroplasticity deficits could be reversed by pharmacologic intervention. The D-serine dosing regimen produced a 3-fold elevation in serum D-serine levels in SR−/− mice as compared to WT mice, which completely normalized D-serine content in the hippocampus of SR−/− mice and increased cortical D-serine to 70% of WT levels. Not only did chronic D-serine administration rescue the LTP impairments in SR−/− mice, but it also reversed the deficits in BDNF protein expression and downstream signaling cascades, including p-Akt and p-mTOR. Furthermore, this dosing regimen did not cause nephrotoxicity.
Conclusions: These data demonstrate that D-serine is necessary for the induction of LTP at the PP-DG synapses and critical for Akt/mTOR signaling. As the reductions in Akt/mTOR signaling were present in the synaptoneurosome fraction, this finding suggests that local dendritic protein translation is reduced in SR−/− mice, consistent with the reduced spine density and impaired LTP. Our findings highlight the convergence of multiple schizophrenia susceptibility pathways, including BDNF, TrkB, Akt, and miR-132 in an animal model of constitutive NMDAR hypofunction. Furthermore, the ability of peripherally administered D-serine to correct electrophysiological and biochemical abnormalities in the hippocampus provides support for the use of D-serine as a treatment of NMDAR hypofunction and its consequent synaptic pathology in schizophrenia.
Keywords: D-serine, schizophrenia, NMDA receptor, Akt, mTOR, LTP
Disclosure: D. Balu, Nothing to Disclose; Y. Li, Nothing to Disclose; M. Puhl, Nothing to Disclose; V. Bolshakov, Nothing to Disclose; J. Coyle, **Part 1:** I am a consultant for Biovail, Puretech, Abbott, and Bristol-Myers Squibb, and I own stock in Abbott. A patent owned by Massachusetts General Hospital for the use of D-serine as a treatment for serious mental illness could yield royalties for myself.

M10. Inflammatory Th17 Cells Promote Depression-like Behavior in Mice

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Background: Recognition of substantial immune-neural interactions is revising dogmas about their insular actions and revealing that immune-neural interactions can substantially impact CNS functions. The inflammatory cytokine interleukin-6 promotes susceptibility to depression and drives production of inflammatory T helper 17 (Th17) T cells, raising the hypothesis that in mouse models Th17 cells promote susceptibility to depression-like behaviors.
Methods: Behavioral characteristics were measured in male mice administered Th17 cells, $CD4^+$ cells, or vehicle, and in $ROR\gamma T^{+/GFP}$ mice or male mice treated with RORγT inhibitor or anti-IL-17A antibodies.
Results: Mouse brain Th17 cells were elevated by learned helplessness and chronic restraint stress, two common depression-like models. Th17 cell administration promoted learned helplessness in 89% of mice in a paradigm where no vehicle-treated mice developed learned helplessness, and impaired novelty suppressed feeding and social interaction behaviors. Mice deficient in the RORγT transcription factor necessary for Th17 cell production exhibited resistance to learned helplessness, identifying modulation of RORγT as a potential intervention. Treatment with the RORγT inhibitor SR1001, or anti-IL-17A antibodies to abrogate Th17 cell function, reduced Th17-dependent learned helplessness.

Conclusions: These findings indicate that Th17 cells are increased in the brain during depression-like states, promote depression-like behaviors in mice, and specifically inhibiting the production or function of Th17 cells reduces vulnerability to depression-like behavior, suggesting antidepressant effects may be attained by targeting Th17 cells.
Keywords: T cells, depression, lithium, RORgammaT, Th17
Disclosure: E. Beurel, Nothing to Disclose; L. Harrington, Nothing to Disclose; R. Jope, Nothing to Disclose.

M11. Effects of Baclofen and Naltrexone, Alone and in Combination, on Binge Eating in Rats

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Background: Binge eating has similarities to drug abuse, and pharmaceutical treatments used to treat drug addiction can also affect binge eating. Specifically, the GABA agonist baclofen (BAC) can reduce intake of some drugs of abuse as well as fat, and naltrexone (NAL), an opioid antagonist, can suppress alcohol and food intake. The present study tested the effect of NAL-BAC combinations on binge intake of fat- and sugar-rich diets.
Methods: Male Sprague-Dawley rats (n = 10/group) were maintained on binge access to standard chow and a 10% sugar solution, 10% sugar-35% fat emulsion, or 35% fat emulsion, for 21 days. The rats were then given intraperitoneal injections of NAL (0.1 or 1.0 mg/kg), BAC (1.0 or 1.8 mg/kg) and NAL-BAC (0.1 mg/kg NAL and 1.0 mg/kg BAC, or 1.0 mg/kg NAL and 1.8 mg/kg BAC).
Results: NAL-BAC (1.0 and 1.8 mg/kg, respectively) suppressed binge eating. Cohen's *d* revealed that NAL-BAC was more effective in decreasing intake than either drug alone, both 1 and 12 h after injection. Similarly, BAC (1.8 mg/kg) suppressed intake in the fat and sugar-fat groups up to 12-h post injection, but did not affect intake in the sugar group. NAL alone did not suppress intake, and none of the drugs decreased chow intake.
Conclusions: These results suggest that the combination of BAC and NAL is superior to either drug alone in suppressing binge intake of palatable foods rich in fat and/or sugar, and this combination might be a useful therapeutic tool for patients who binge eat.
Keywords: binge eating, palatable foods, fat, sugar, addiction
Disclosure: N. Avena, Nothing to Disclose; M. Gold, Nothing to Disclose.

M12. Cariprazine Exhibits Dopamine D3 Receptor-Dependent Antidepressant-Like Activity in the Chronic Unpredictable Stress Model of Anhedonia

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Background: Depression is a common, chronic, recurring illness associated with significant functional and social impairment. Effective treatment of depression is challenging, with most patients failing to achieve remission after initial antidepressant treatment. As a result, atypical antipsychotics are increasingly being used to augment antidepressant efficacy in patients with depression. Cariprazine, a D_3-preferring D_3/D_2 dopamine receptor partial agonist, is a candidate antipsychotic in late stage clinical development for the treatment of schizophrenia and bipolar mania. The *in vitro* affinity of cariprazine for the dopamine D_3 receptor is approximately one order of magnitude greater than for the D_2 receptor. In previous rodent studies, cariprazine showed a more balanced D_2 and D_3 receptor brain occupancy *in vivo* compared to other antipsychotics, which displayed preferential occupancy for D_2 vs D_3 receptors. Furthermore, cariprazine was also found to be unique in its ability to increase D_3 receptor levels, in D_3 receptor-rich brain regions, following chronic treatment. Since the D_3 receptor is thought to be involved in the regulation of mood, a compound that exhibits high affinity and occupancy of D_3 receptors may be effective as a treatment for depressive disorders as well as the negative symptoms of schizophrenia. Cariprazine has demonstrated significant antidepressant-like activity in a chronic mild stress model in rats by reducing anhedonic behavior, a hallmark symptom of depression and negative symptoms of schizophrenia. However, the mechanism of action for cariprazine's antidepressant-like effects had not been elucidated. The objectives of the current study were to investigate the antidepressant-like effects of cariprazine in a chronic unpredictable stress (CUS) model in mice and determine whether this effect is mediated by the D_3 receptor.
Methods: To induce anhedonia, male C57BL6 mice were exposed to a variable sequence of unpredictable stressors (2/day) for a total of 26 days. During the study period, mice were administered intraperitoneal (IP) saline, cariprazine, or imipramine (20 mg/kg QD as a positive control); control mice received saline but were not exposed to the CUS procedure. After 21 days of CUS and drug/saline treatment, the hedonic-like state of each animal was assessed by measuring consumption of a 1% sucrose solution. In the first phase of the experiment, cariprazine (0.03, 0.1, and 0.2 mg/kg, BID) was tested in wild-type (WT) mice in the CUS-induced anhedonia model. In the second phase of the experiment, cariprazine (0.1 and 0.2 mg/kg, BID) was tested in D_3 receptor knockout (D_3-KO) mice in parallel with WT mice to determine if the antidepressant activity of cariprazine in this model is mediated through the D_3 dopamine receptor. For all experiments, water consumption and locomotor activity were evaluated to control for changes in overall liquid consumption or ambulatory behavior. One-way ANOVA analysis was used to determine whether CUS produced significant effects on sucrose consumption. Two-way ANOVA was used to test for significant interactions between genotype and treatment. Pairwise comparison using Fisher's protected least significant difference (PLSD) was performed to compare treatment groups.
Results: In the first phase of the study, following 21 days of CUS, saline-treated WT mice exhibited a significant decrease in sucrose consumption, indicating CUS-induced anhedonia ($P < .0001$). Cariprazine 0.2 mg/kg (but not 0.03 or 0.1) and imipramine both demonstrated antidepressant activity by significantly attenuating the CUS-induced effect on sucrose consumption (both treatments, $P < .0001$ vs CUS + saline). Water consumption and locomotor activity were similar between treatment groups, indicating that effects on sucrose consumption were not due to changes in drinking behavior or overall ambulatory activity. In the second phase of the study, D_3-KO mice, similar to WT mice, exhibited decreases in sucrose consumption following CUS ($P < .0001$). Similar to the previous experiment, the CUS-induced decrease in sucrose consumption was attenuated by both cariprazine (0.2 mg/kg BID) and imipramine treatment in WT mice (both $P < .0001$ versus CUS + saline). However, in the D_3-KO mice, cariprazine did not inhibit CUS-induced decreases in sucrose consumption ($P = .556$), suggesting that the antidepressant activity of cariprazine is mediated through the D_3 receptor. There were no significant effects of CUS, cariprazine treatment, or genotype on water consumption or locomotor activity.
Conclusions: Cariprazine, similar to imipramine, demonstrated significant antidepressant-like activity by attenuating CUS-induced anhedonia in WT mice. However, this effect was not observed in D_3-KO mice, indicating that dopamine D_3 receptors are required to mediate the antidepressant-like effects of cariprazine. These data suggest that cariprazine with its unique dopamine D_3 receptor-preferring mechanism of action may have potential efficacy in the treatment of depressive disorders and negative symptoms of schizophrenia.

Keywords: animal model, cariprazine
Disclosure: R. Duman, **Part 1:** Received speaker fees and research grants from Forest Laboratories, Inc., **Part 4:** Received speaker fees and research grants from Forest Laboratories, Inc.; V. Duric, Nothing to Disclose; M. Banasr, Nothing to Disclose; N. Adham, **Part 1:** Full time employee of Forest Research Institute., **Part 2:** Full time employee of Forest Research Institute., **Part 3:** Full time employee of Forest Research Institute; B. Kiss, **Part 1:** Full time employee of Gedeon Richter, Plc., **Part 2:** Full time employee of Gedeon Richter, Plc., **Part 3:** Full time employee of Gedeon Richter, Plc.; I. Gyertyan, **Part 1:** Full time employee of Gedeon Richter, Plc., **Part 2:** Full time employee of Gedeon Richter, Plc., **Part 3:** Full time employee of Gedeon Richter, Plc.

M13. The Peripheral Immune System Functionally Contributes to Susceptibility to Repeated Social Defeat Stress

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Background: Subjects with major depression have increased circulating levels of pro- inflammatory cytokines, such as Interleukin-6 (IL-6), which is thought to reflect hyperactivity of their peripheral immune system (Dowlati et al., 2009). We have previously shown similar increases in serum IL-6 levels following repeated social defeat stress (RSDS), a mouse model of mood and anxiety disorders. Mice that are susceptible to RSDS initially have an exaggerated release of IL-6 and exhibit sustained increases of IL-6 levels for at least 1 month following the last defeat. Thus, we predict that there are innate differences in the immune response to stress in susceptible mice that drives depression- and anxiety-like behavioral phenotypes.
Methods: We used RSDS to examine individual differences in response to stress; some animals termed susceptible show a spectrum of depression-like behavior, whereas resilient animals are more similar to controls. To determine whether IL-6 release can be used as a predictive biomarker, we isolated and cultured peripheral blood mononuclear cells (PBMCs) prior to exposure to RSDS, stimulated with the endotoxin lipopolysaccharide (LPS), and then measured IL-6 using enzyme linked immunosorbent assays (ELISA). To determine if peripheral IL-6 was necessary for the development of susceptibility to RSDS, we systemically injected a separate group of animals with an antibody that neutralizes IL-6 in the periphery and tested them for social avoidance, anhedonia (sucrose preference) and anxiety (elevated plus maze). To examine whether the peripheral immune system was sufficient to functionally drive these behavioral adaptations to RSDS, we first ablated the peripheral immune system of naïve mice using irradiation. We then replaced their immune system with bone marrow either from a susceptible mouse following 10 days of RSDS, or a control mouse with a little or no IL-6 response to endotoxin challenge. We then exposed mice to a sub-threshold micro-defeat and tested for depression and anxiety-like behavior.
Results: PBMCs isolated prior to social defeat from mice that later developed a susceptible phenotype had a larger release of IL-6 following LPS stimulation compared to mice that went on to become resilient. Systemic injections of an IL-6 neutralizing antibody in the periphery blocked RSDS-induced social avoidance and anhedonia. Finally, mice that received bone marrow transplants from a susceptible mouse showed greater social avoidance, anhedonia, and anxiety-like behavior following a sub-threshold micro-defeat.
Conclusions: These studies indicate that the peripheral immune system contributes to the development of susceptibility to social defeat stress. We found that a hyperactive peripheral immune response to stress is a risk factor for the development of depression and anxiety-like behavior. We also show a direct functional role of the peripheral immune response to stress in regulating depression and anxiety like behaviors. Together these studies indicate that innate differences in the immune responses to stress may underlie the development of depression like behavior and serve as a novel therapeutic target for treatment.
Keywords: Depression, cytokines, stress, neuroimmunology, anxiety.
Disclosure: G. Hodes, Nothing to Disclose; S. Golden, Nothing to Disclose; D. Christoffel, Nothing to Disclose; M. Pfau, Nothing to Disclose; M. Heshmati, Nothing to Disclose; M. Leboeuf, Nothing to Disclose; M. Merad, Nothing to Disclose; S. Russo, **Part 4:** Subcontract with Johnson & Johnson to study antidepresseant efficacy of anti-IL6 compounds.

M14. Distinct CRF Protein Expression Patterns in Two Different CRF Overexpressing Mouse Models

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Background: Corticotropin-releasing factor (CRF) orchestrates the stress response. CRF is elevated in cerebrospinal fluid of patients with depression, post-traumatic stress disorder, and childhood trauma, implicating CRF hypersecretion in the development of stress-related psychiatric disorders. To better understand the biological consequences of CRF hypersecretion, independent lines of CRF-overexpressing mice were developed. The first line overexpresses CRF constitutively starting at birth. Other more recent lines have CRF overexpression restricted to developmental time windows, brain regions, or cell types. Although CRF mRNA expression has been evaluated in several of these models, CRF immunoreactivity throughout the brain has not been examined or compared between models. Here we compared the distribution of CRF immunoreactivity in mice with constitutive CRF overexpression from birth to mice with transient forebrain overexpression over different developmental time points.
Methods: Two different CRF overexpressing mouse models were used. The first model carries a chimeric CRF transgene comprising the methallothionein promoter driving the rat CRF gene and backcrossed onto C57BL/6 mice (Stenzel-Poore et al., 1992). These CRF transgenic (CRF-Tg) mice were derived and purchased from Jackson laboratories along with wild type (WT) littermates. The second model carries a rat CRF transgene under the control of a tet operator (tet-O) and the rtTA2 transcription factor gene driven by the CaMKIIα promoter and backcrossed onto C57BL/6 mice. Double mutants (CRF-cam) have forebrain-specific CRF overexpression induced by doxycycline (DOX) ("rtTA2-ON" system). These transgenes allow the induction of CRF overexpression by DOX administration in food at three developmental stages: juvenile (P2-P23), adulthood (P70-P90), and lifetime (P2-P90). Control subjects are double mutants not treated with DOX. To compare the distribution of CRF expression, adult (>90 days) WT and overexpressing mice from both models were sacrificed and brain sections were processed to visualize CRF immunoreactivity using immunofluorescence. A separate set of CRF-cam mice was behaviorally evaluated using startle reactivity, prepulse inhibition, and the light-dark box.
Results: CRF-Tg mice first were compared to CRF-cam mice with lifetime overexpression. Although the duration of overexpression was similar in both models, the distribution of CRF immunoreactivity was distinct. In CRF-Tg mice, CRF immunoreactivity was observed in similar regions as in WT mice including the bed nucleus of the stria terminalis (BNST), hypothalamus, amygdala, dorsal raphe, substantia nigra pars compacta, ventral tegmental area, and the locus coeruleus region (peri-LC). As expected, CRF immunolabeling was higher in these regions in CRF-Tg than WT

mice. In contrast, CRF-cam mice with lifetime overexpression had robust CRF immunoreactivity in basal ganglia-related structures, including the striatum, globus pallidus, and substantia nigra pars reticulata. CRF immunoreactivity was more prominent in the dentate gyrus and CA3 region of the hippocampus in lifetime CRF-cam mice than in other groups. In contrast to CRF-Tg mice, CRF immunoreactive fibers were sparse in the hypothalamus, dorsal raphe and peri-LC. CRF-cam mice with overexpression during different developmental time points also were compared. The pattern of CRF-immunoreactivity in adulthood only CRF-cam mice was similar to that seen in lifetime CRF-cam mice. Notably, the distribution of CRF-immunoreactivity in adult CRF-cam mice with juvenile overexpression showed a closer resemblance to CRF-Tg mice, i.e., greater CRF immunolabeling in the BNST, dorsal raphe and peri-LC, without the striatal CRF immunolabeling observed in their CRF-cam counterparts. Differences in the pattern of CRF-immunoreactivity between CRF-cam mice that overexpress CRF only in the juvenile period compared to those that overexpress throughout life or in adulthood only may reflect compensatory mechanisms. Behaviorally, CRF-cam mice with juvenile and lifetime overexpression showed similar changes in startle reactivity such as decreased startle habituation and decreased prepulse inhibition in females. Interestingly, this change was more intense in the juvenile group in which avoidance in the light-dark box was further increased in females. In contrast, behavior was not altered in mice with adulthood overexpression.

Conclusions: This study revealed unique patterns of CRF overexpression in different transgenic mouse models. CRF-Tg and CRF-cam mice with juvenile CRF overexpression best represent CRF overexpression in brain regions in which CRF is typically present. CRF-cam mice that overexpress CRF throughout life or only in adulthood exhibit an atypical pattern of CRF expression that involves basal ganglia-related structures. Notably, CRF overexpression limited to the juvenile period was associated with increased anxiety behaviors and sensorimotor gating, selectively in females, indicating that CRF hypersecretion in early life may be a risk factor for stress-related disorders. Finally, these data underscore the importance of the distribution of CRF expression in the interpretation of data and in the choice of the appropriate genetic model.

Keywords: anxiety stress sex difference

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M15. Risk-taking in Adolescence: Relationships with Cocaine Self-administration and Involvement of Dopamine Signaling

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Background: Elevations in risk-taking are characteristic of both adolescence and drug abuse, but the relationships among risk-taking, adolescence, and drug abuse are difficult to disentangle in humans. Here we used a rat model of risky decision making (the Risky Decision-making Task, RDT) to assess relationships between adolescent risk-taking and cocaine self-administration. In addition, we used analyses of mRNA expression and behavioral pharmacology to characterize the involvement of dopamine signaling in risk-taking.

Methods: In Experiment 1, adolescent male Long-Evans rats (P25) were trained in the RDT, in which they were given choices between two response levers, the first which delivered a small (1 pellet), "safe" food reward and the second which delivered a large (3 pellets), "risky" food reward accompanied by the risk of a mild footshock, the probability of which increased over the course of the test session in consecutive blocks of discrete trials (0, 25, 50, 75, 100%). Upon completion, half of the rats were implanted with i.v. jugular catheters and after recovery, were allowed to self-administer 0.5 mg/kg/infusion cocaine for 2h/day for 5 days, followed by 1.0 mg/kg/infusion for 6h/day for 14 days. The other half of the rats self-administered an oral sucrose solution to control for instrumental learning experience. Upon completion of self-administration, rats remained abstinent from cocaine (or sucrose) for 3 weeks before being retested in the RDT for 4 weeks. In Experiment 2, adolescent rats were characterized in the RDT, followed by sacrifice for *in situ* hybridization analyses of D1 and D2 dopamine receptor expression in striatal subregions. In Experiment 3, adolescent rats were characterized in the RDT, followed by assessment of the effects of microinjections of the D2-like agonist quinpirole directly into dorsal or ventral striatum.

Results: In Experiment 1, there were substantial individual differences in adolescent rat performance in the RDT, such that some rats preferred the large, risky reward whereas others preferred the small, safe reward. This individual variability predicted cocaine intake during acquisition of cocaine self-administration, such that greater preference for the large, risky reward (greater risk-taking) was associated with greater cocaine intake. In addition, following self-administration and 7 weeks of abstinence, rats that self-administered cocaine showed significantly elevated risk-taking compared to both sucrose controls and their performance during adolescence. In Experiment 2, there were significant inverse correlations between risk-taking in adolescence and D1 mRNA in dorsomedial striatum and D2 mRNA in dorsolateral striatum and nucleus accumbens shell, such that greater choice of the large, risky reward (more risk-taking) was associated with lower levels of dopamine receptor mRNA expression. In Experiment 3, microinjection of quinpirole into ventral (but not dorsal) striatum caused a dose-dependent decrease in preference for the large, risky reward (less risk-taking).

Conclusions: Data from these experiments indicate that elevated risk-taking in adolescence is predictive of future acquisition of cocaine self-administration, and that cocaine self-administration, in turn, causes elevations in risk-taking that last well into abstinence. Elevated risk-taking in adolescence is also associated with low levels of striatal dopamine (particularly D2) receptor mRNA, consistent with previous work across species which has linked low levels of striatal D2 receptor availability with cocaine self-administration. Considered together, these data suggest that attenuation of striatal dopamine receptor activity, particularly during early development, may be a feature of several forms of maladaptive behavior, and furthermore that targeting this attenuation may hold promise for reducing such behaviors.

Keywords: addiction, decision making, risk taking, cocaine, rat

Disclosure: B. Setlow, Nothing to Disclose; M. Mitchell, Nothing to Disclose; V. Weiss, Nothing to Disclose; S. Beas, Nothing to Disclose; D. Morgan, Nothing to Disclose; J. Bizon, Nothing to Disclose.

M16. The Hyperactive (HYPER) Rat: A Potential Animal Model of Bipolar Disorder

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Background: Development of new treatments for bipolar disorder (BD) has been hindered by the unavailability of animal models for their discovery and testing. We describe here a rat line that shows behavioral and physiological characteristics similar to what humans with BD show; moreover, this rat appears to embody genetic abnormalities that are also similar to human BD. This is the first animal that shows such similarities to human BD.
Methods: In all experiments in which ambulatory activity was recorded, animals were singly housed in standard colony cages directly on bedding with access to ad libitum food and water, and activity was recorded around the clock by interruption of photocell beams mounted externally along the long axis of the colony cage. Activity for each rat was typically recorded for 10-14 days, but in some experiments for up to 3.5 months. In some experiments, electric shock was delivered to rats; shock was given outside of the activity cage and was delivered via electrodes affixed to the rat's tail. Selective breeding (used in derivation of the rat line, and continuation of the line) was carried out by measuring ambulatory activity and pairing males and females that showed desired activity patterns. Anxiety of animals was assessed using an elevated plus maze and/or open field. Assessment of activity in dopaminergic brain regions as done by measuring c-fos expression as well as by measuring concentration of dopamine and relevant metabolites post mortem in dopaminergic cell-body regions and in dopamine-rich forebrain regions.
Results: The rat line described here was generated from a single litter of rats all of which were discovered to be hyperactive in the home cage (i.e., showed higher-than-normal ambulatory activity). After brother-sister breeding of these initial progenitor rats, their offspring, now for 50 generations, have remained distinctly hyperactive. This rat line was named the Hyperactive (HYPER) rat. Salient observations of the HYPER rat related to BD have been: first, early-on we observed that HYPER responded to stress with an outburst of extreme hyperactivity (mania?) that began 2-3 days after the stressful event (e.g., administration of tail shock) and lasted 4-7 days. Later, we observed that female HYPER rats in particular (but also some male rats as well) would, following tail shock, "cycle" between periods of hyperactivity and depressed activity after stress when measurement is carried out for a long period of time (3-4 months). This is the first animal discovered that shows "cycling" between hyperactivity and hypoactivity as in human BD. Other characteristics of HYPER rats found in human BD are (a) elevated anxiety and (b) reduced dopaminergic function in forebrain regions. Also, a small number of HYPER rats show kerototic skin lesions similar to the dermatological disorder Darier's disease in humans that has been found to co-segregate with human BD; this suggests similar genetic abnormalities in the HYPER rat and in human BD.
Conclusions: The HYPER rat thus shows similarity to human BD with respect to behavioral symptoms and underlying pathophysiology, and, quite possibly, similar genetic determinants as are present in humans with BD. Study of this animal model may help development of new treatments for BD.
Keywords: bipolar disorder, stress, depression, animal model, activity
Disclosure: J. Weiss, Nothing to Disclose; K. Boss-Williams, Nothing to Disclose.

M17. Opposite Effects of Tolcapone on Amphetamine-disrupted Startle Gating in Low vs. High COMT-expressing Rat Strains

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Background: The dopaminergic regulation of sensorimotor gating in rats – as measured by prepulse inhibition (PPI) of the startle reflex - is used to understand the biology of deficient gating in brain disorders such as schizophrenia. Differences in sensitivity to the PPI-disruptive effects of dopamine (DA) agonists in Sprague-Dawley (SD) vs. Long Evans (LE) rats are heritable, reflect differential activation of DA signaling in the nucleus accumbens (NAC), and are associated with differences in the expression of specific NAC genes. Among these differences in gene expression, LE > SD expression of the mRNA for the catecholamine catabolic enzyme, COMT, is detected in several brain regions that regulate PPI. In humans, both basal and drug-modified PPI differs significantly between individuals with polymorphisms conferring low- vs. high-activity of COMT. Here, we used the COMT inhibitor, tolcapone, to assess the role of COMT activity in regulating the differential effects of the DA releaser, amphetamine (AMPH), on PPI in SD and LE rats.
Methods: Acoustic startle and PPI were assessed in 16 SD and 16 LE male rats after pretreatment with tolcapone (vehicle vs. 30 mg/kg ip; 80 min prior to testing) and treatment with AMPH (vehicle vs. 4.5 mg/kg sc; 10 min prior to testing), using 10–120 ms prepulse intervals.
Results: ANOVA revealed significant interactions of AMPH dose x strain ($p < 0.007$) and AMPH dose x strain x tolcapone dose ($p < 0.003$). After tolcapone (30 mg/kg) pretreatment, AMPH significantly potentiated PPI in LE rats ($p < 0.01$), and significantly disrupted PPI in SD rats ($p < 0.04$), when PPI was collapsed across all prepulse intervals. Tolcapone effects appeared to be both strain- and temporally-specific: in LE rats, tolcapone potentiated AMPH's PPI-enhancing effects at short-intervals and blocked AMPH's PPI-disruptive effects at long intervals. In SD rats, tolcapone had the opposite effects: it blocked AMPH's short-interval effects on PPI, and potentiated its long interval effects. These patterns could not be explained by drug effects on pulse alone startle magnitude.
Conclusions: In this experiment, the impact of COMT inhibition on AMPH-modified PPI was categorically different in strains exhibiting low vs. high levels of forebrain COMT expression. COMT inhibition blocked the gating-disruptive effects of AMPH in high COMT-expressing LE rats, and potentiated the gating-disruptive effects of AMPH in low COMT-expressing SD rats. These findings are consistent with reports in humans that tolcapone has opposite effects on PPI among individuals with polymorphisms conferring low vs. high COMT activity. The present model thus provides a basis for understanding the mechanisms by which the effects of COMT inhibition on sensorimotor gating – and potentially, related neurocognitive and clinical functions - under hyperdopaminergic states are dependent on an individual's basal levels of COMT activity.
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Keywords: amphetamine, catechol-O-methyltransferase, prepulse inhibition, schizophrenia, strain
Disclosure: M. Breier, Nothing to Disclose; S. Hines, Nothing to Disclose; S. Herrera, Nothing to Disclose; M. Weber, **Part 1:** Full time employee, Genentech, Inc., **Part 2:** Full time employee, Genentech, Inc., **Part 3:** Full time employee, Genentech, Inc., N. Swerdlow, **Part 1:** Neurocrine, Inc. (Consultant).

M18. Astrocyte-specific Ablation in the Mouse Prefrontal Cortex Induces Depressive-like and Anxiety-like Deficits

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Background: Growing evidence implicates glia in the pathophysiology of depression. Reductions in the number of astrocytes have been reported in postmortem studies examining brain tissue from patients with major depression. Preclinical studies have confirmed some of these changes in both the hippocampus and the prefrontal cortex (PFC) in rodent models of depression based on chronic stress. More specifically we have reported that chronic unpredictable stress reduced number of astrocytes expressing GFAP (glial fibrillary associated protein) and others have emonstrated that the S100Beta-positive cell population was unaffected. We have also demonstrated that rat prefrontal cortex (PFC) glial ablation using local infusion of a gliotoxin induces behavioral deficits similar to chronic stress, including anhedonia, anxiety and helplessness. However, the specific contribution of each subtype of glial cell in the development of depressive-like symptoms remains to be characterized.

Methods: To answer this question, we examined the behavioral consequences of targeted ablation of GFAP-positive cells in the PFC in baseline and stress conditions on anhedonia, anxiety and helplessness. To achieve this goal, we developed an approach adapted from *cre/loxP system* strategy in which GFAP-positive cells of the PFC are altered to express the diphtheria toxin (DT) receptor (DTR), and thereby made sensitive to DT exposure. Adult GFAP-*cre* mice and wild type (WT) littermates were infused with AAV5 virus in the PFC. The viral construct was design to express DTR only in *cre* expressing cells; more specifically the *loxp* sequences were strategically positioned around the CMV promoter to induce the flipping of the promoter thereby inducing expression of DTR in *cre*-cells. Three weeks after bilateral infusion of the virus (AAV5-fCMV-DTR) in the PFC, animals were injected i.p. daily with saline or DT at 3 different doses (0.1, 5, 20 ug/kg) for 4 days and daily sucrose (1%) consumption over 24h-period was measured. When an effect on sucrose consumption was observed, we also analyzed the effect of glial ablation in other behavioral tests known to be affected by stress and antidepressant treatment.

Results: Two days after the first injection of DT, GFAP*cre* + AAV5-fCMV-DTR mice injected with 20 ug/kg of DT showed a significant decrease in sucrose consumption when compared with GFAP*cre* + AAV5-fCMV-DTR mice injected with saline or the 0.1 ug/kg DT. On day 3, both 5 and 20 ug/kg GFAP*cre* + AAV5-fCMV-DTR mouse groups showed reduced sucrose consumption when compared with the saline or the low DT dose group. WT mice infused with AAV5-fCMV-DTR showed no change in sucrose consumption when injected with saline or the different doses of DT. We also measured water consumption on day 5 and found no significant difference in WT or GFAP*cre* mice injected with saline or the various doses of DT. We found that animals GFAP*cre* + AAV5-fCMV-DTR injected with 5 and 20 ug/kg still showed decreased sucrose consumption on day 8, but not day 14 (4 or 10 days after the last injection of DT, respectively). We also examined the consequences of the cell ablation in behaviors measuring the anxiety-like state of the animals. Overall, we found that although the effects were not always significant, the GFAP*cre* + AAV5-fCMV-DTR animals treated with DT tend to exhibit more anxiety-like deficits when compared to the GFAP*cre* + AAV5-fCMV-DTR animals injected with saline. More precisely, we found that GFAP*cre* + AAV5-fCMV-DTR animals treated with the 3 doses of DT showed a significant increase in their latency to drink a milk solution in the novelty induced hypophagia test ($P < 0.05$), a trend toward increased latency to feed in the novelty suppressed feeding test ($P = 0.15$) and a trend to spend less time in the center in the open field test ($P = 0.13$).

Conclusions: Our results demonstrate that selective ablation of GFAP-positive cells in the PFC induces rapid anhedonia- and anxiety-like deficits that persist for at least 8 days but are transitory and not observed at day 14; this reversal could be due to glial renewal after cessation of DT infusion, a possibility that we are currently testing. These findings demonstrate that loss of GFAP-positive cells in the PFC is sufficient to cause depressive behavior, supporting the hypothesis that glial loss in depressed patients contributes to depressive symptoms. Moreover, this cell selective ablation approach will allow us to further study the cellular consequences of this astrocyte-specific cortical ablation on the function of the PFC, as well as the contribution of other populations of cells (glial or neuronal) in the development of depressive-like behavior.

Keywords: Depression Animal models Astrocytes anhedonia anxiety

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M19. Optogenetic Control of Serotonergic Neurons and Anxiety-like Behavior

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Background: It has generally been thought that serotonin release in the forebrain attenuates anxiety. However, there is so far no direct evidence proving this hypothesis because there has been no method that reversibly, selectively, and temporally-specifically controls serotonergic activity. Although there is extensive indirect evidence, it is mixed. For example, while selective serotonin reuptake inhibitors (SSRIs) are first-line agents for anxiety disorders, increased anxiety is often observed during the acute phase of treatment. Therefore, in the present study, we aimed to obtaining direct evidence about the causal relationship between serotonin and anxiety using recently developed optogenetic tools.

Methods: We obtained transgenic mice expressing channelrhodopsin-2 (ChR2) mutant (C128S) only in central serotonergic neurons by crossing tetO-ChR2(C128S)-EYFP knock-in mice with Tph2-tTA BAC transgenic mice. The activation/deactivation rates of C128S mutant with blue light are slow ($\tau_{on} = 20$ ms, $\tau_{off} = 108$ s). We inserted an optical fiber to the median raphe nucleus (MRN). We applied blue light to open ChR2, and measured extracellular serotonin levels in the ventral hippocampus and recorded behavioral changes in the elevated plus maze. Yellow light was used as a negative control because it will not open ChR2.

Results: We demonstrated that blue light illumination to the MRN significantly increased extracellular levels of serotonin in the ventral hippocampus while yellow light did not. Moreover blue light illumination affected anxiety-like behavior in the elevated plus maze while yellow light did not.

Conclusions: Thus we obtained direct evidence of the causal relationship between serotonergic activity in the MRN and anxiety.
Keywords: optogenetics, anxiety, 5-HT, raphe
Disclosure: Y. Ohmura, Nothing to Disclose; K. Tanaka, Nothing to Disclose; A. Yamanaka, Nothing to Disclose; M. Yoshioka, Nothing to Disclose.

M20. Depressive-like Phenotype in Transgenic Mice with CNS Overexpression of IL-6

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Background: Recent clinical data implicate cytokines in the pathophysiology of major depressive disorder (MDD). IL-6 is a pleiotropic inflammatory cytokine that has been reported to be elevated in patients diagnosed with MDD and can be normalized with successful antidepressant treatment (Lanquillon et al 2000; Basterzi et al 2005). Intriguingly, in patients who fail to respond to antidepressant therapy, IL-6 levels remain elevated (O'Brien et al 2007). Previous data from our laboratory have demonstrated a robust depressive-like phenotype following central administration of recombinant mouse IL-6 as well as increases in IL-6 levels in cortex homogenates of rodents subjected to stress (Sukoff Rizzo et al 2012 in revision). Moreover, we have demonstrated that the antidepressant-like effects of fluoxetine are attenuated in the presence of centrally administered IL-6 as well as in the LPR mouse model which demonstrates endogenous overexpression of IL-6 in the CNS and a depressive-like phenotype. In order to further test our hypothesis of the involvement of IL-6 in depression, we evaluated the behavioral phenotype of transgenic mice with overexpression of IL-6 in the CNS (GFAP IL-6).
Methods: All animal studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the NIH (Pub. 85-23, revised 1996) and were approved by Pfizer's Institutional Animal Care and Welfare Committees. The generation and production of these mice have been described in detail previously (Campbell et al 1993). Briefly, IL-6 expression in the CNS was targeted to astrocytes via a GFAP promoter. Male mice heterozygous (HET) for the IL-6 transgene were used as breeder mice (C57BL/6J background strain). Male HETs were bred to normal C57BL/6J females to produce an F1 generation, and subsequently backcrossed (HET x HET), resulting in mice homozygous (HOM) for the IL-6 transgene and WT littermate controls. WT, HET, and HOM male and female mice (age 9-11 weeks at testing) were initially evaluated in the tail suspension test followed by the open field test with a 3 day inter-testing interval. A second cohort of behaviourally naive male WT and HOM mice (age 10-12 weeks at testing) were evaluated in the tail suspension test to confirm the behavioural effects observed in the initial study.
Results: Consistent with the hypothesis that increased immobility time is indicative of depressive-like behavior, male HOM mice (9-11 weeks of age) demonstrated statistically significant increases in immobility time as analyzed by one-way ANOVA [$F(2,30) = 11.36$; $p < 0.001$] with a Dunnett's *post hoc* test demonstrating a statistically significant increase in male HOM mice relative to WT littermate controls ($p < 0.001$). Importantly, there were no significant alterations in general exploratory activity as measured by total distance in an open field in male HOM mice relative to WT littermate controls [$F(2,28) = 0.010$; $p = 0.99$]. Although female HOM mice also demonstrated modest, increases in immobility time relative to WT littermate controls [$F(2,26) = 3.45$; $p < 0.05$], female HOM mice also demonstrated significant reductions in general exploratory behaviours [$F(2,29) = 19.32$; $p < 0.0001$]. In a separate study, evaluation of a second cohort of male WT and HOM mice in the tail suspension test confirmed the significant increases in immobility time ($p < 0.01$), consistent with a depressive-like phenotype.
Conclusions: The depressive-like phenotype of HOM GFAP IL-6 mice is in line with our previous data demonstrating that central administration of IL-6 produces increases in immobility time in the tail suspension and forced swim tests in Swiss Webster mice, indicative of a depressive-like effect (Sukoff Rizzo et al 2012 in revision). The behavioural phenotype of the HOM mice is also consistent with the behavioural phenotype of the LPR mouse model with endogenous overexpression of IL-6 in the CNS. Given the robust depressive-like phenotype in male HOM mice, future studies will aim to employ the GFAP IL-6 mice as a model for treatment resistant depression, and evaluate the ability of novel compounds, targeting IL-6 and the JAK/STAT pathway, to produce antidepressant efficacy.
Keywords: treatment resistant depression, IL-6, cytokines, animal models
Disclosure: S. Sukoff Rizzo, **Part 1:** I am a full time employee of Pfizer, **Part 4:** Pfizer provided full financial support of my PhD work (part-time) while I was a full time employee with the company; Z. Hughes, **Part 1:** Full time employee of Pfizer; S. Neal, **Part 1:** Full time employee of Pfizer; J. Roos, **Part 1:** Full time employee of Pfizer; S. Rosenzweig-Lipson, **Part 1:** Dr. Rosenzweig-Lipson was a full time employee of Pfizer during the inception of this work, AgeneBio - Consultant, VP of Research, Confluence - Consultant, VP of Research, Abbott - Consultant, Shire - Consultant, Vivia - Consultant, UT Galveston - Consultant, **Part 2:** AgeneBio > $10,000 per year, Confluence > $10,000 year, **Part 3:** AgeneBio > 5%, Confluence > 5%, **Part 4:** none; S. Moss, **Part 1:** Dr. Moss received compensation as a member of the scientific advisory board of Pfizer (formerly Wyeth) and has also consulted for GSK, Sepracor, and Sage Therapeutics, **Part 2:** Dr. Moss received compensation as a member of the scientific advisory board of Pfizer (formerly Wyeth) and has also consulted for GSK, Sepracor, and Sage Therapeutics, **Part 3:** Dr. Moss received compensation as a member of the scientific advisory board of Pfizer (formerly Wyeth) and has also consulted for GSK, Sepracor, and Sage Therapeutics, **Part 4:** Dr. Moss received compensation as a member of the scientific advisory board of Pfizer (formerly Wyeth) and has also consulted for GSK, Sepracor, and Sage Therapeutics; N. Brandon, **Part 1:** Dr. Brandon was a full time employee of Pfizer during the inception and execution of this work. Dr. Brandon is currently employed with Astra Zeneca, **Part 2:** Dr. Brandon was a full time employee of Pfizer during the inception and execution of this work. Dr. Brandon is currently employeed with Astra Zeneca as of May 2012, **Part 3:** Dr. Brandon was a full time employee of Pfizer during the inception and execution of this work. Dr. Brandon is currently employeed with Astra Zeneca as of May 2012.

M21. Progress of the Lilly/Pfizer Partnership to Tackle Preclinical Model Development in Psychiatry: Does Chronic ACTH Treatment Render Mice Resistant to Antidepressants?

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Background: Given the existence of antidepressants and anti-psychotics that are generally both efficacious and safe, there are increasing pressures to identify novel agents with greater efficacy (greater response and remission rates) and reduced side-effect liability (e.g. weight gain, sexual dysfunction). These pressures are forcing a fundamentally different approach which is particularly evident in research and development in psychiatry. In 2010, Eli Lilly and Pfizer established a partnership to refine their preclinical research strategies to better predict clinical outcomes. The areas of

focus for this partnership are treatment resistant depression (TRD) and the negative and cognitive symptoms of schizophrenia. Here we describe our efforts in TRD model development to demonstrate how this partnership has leveraged synergies of expertise and experience and opportunities for cost sharing. To help prosecute this approach and assess reproducibility, Psychogenics Inc was brought in as a 3rd independent laboratory with expertise in this area. Our research plan incorporated 2 approaches: 1) Determining whether the CD-1 mouse strain, which has reduced sensitivity to serotonergic antidepressant agents (Witkin et al, ACNP 50th Annual Meeting, 2011) responded to an SNRI and 2) Investigating whether chronic ACTH treatment causes mice to become 'resistant' to antidepressants as has been reported in rats (Kitamura et al, Pharmacol Biochem Behav. 2002;71 (1-2):63-9). To assess whether ACTH treatment had biochemical consequences similar to other chronic stress models, plasma samples were analyzed for markers associated with stress.

Methods: All animal studies were performed in accordance to the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the NIH (Pub. 85-23, revised 1996) and were approved by the relevant Institutional Animal Care and Welfare Committees. 1) Male CD-1 mice (Charles River) were dosed with vehicle, fluoxetine (10-56 mg/kg, i.p.) or duloxetine (5-20 mg/kg, i.p.). Behavior was evaluated in the tail-suspension assay 30 min later where mice were suspended by their tail and immobility time during a 10 min experimental session recorded by a force transducer. 2) Male Balb/cJ (Jackson Labs) mice were implanted with subcutaneous minipumps containing ACTH (0.01 mg/kg/d, s.c. x14d), or vehicle (DMSO 50%). The effects of chronic ACTH administration on immobility time and sensitivity to antidepressants were evaluated in the forced swim test (single 6 min swim session). Mice were dosed with vehicle (water 10 ml/kg i.p.), fluoxetine (15 mg/kg i.p.) or duloxetine (10 mg/kg i.p.) 30 min prior to behavioral testing. To probe the biochemical consequences of chronic ACTH administration, plasma samples were taken from a satellite group of mice and analyzed by ELISA for biochemical markers of chronic stress.

Results: 1) Previous studies by this group (Witkin et al. ACNP, 2011), indicated that of 5 mouse strains tested, CD-1 mice were less sensitive to standard antidepressants such as fluoxetine or imipramine. In these follow-up studies, the resistance of CD-1 mice to the antidepressant-like effects of the SSRI, fluoxetine (10–56 mg/kg; $F_{3,33} = 2.012$; $P = 0.131$), were replicated, however the immobility of this strain was reduced by the SNRI, duloxetine (5-20 mg/kg; $F_{3,32} = 11.8$; $P < 0.001$). 2) Chronic ACTH caused increases in plasma biomarkers associated with chronic stress: corticosterone, insulin and leptin were elevated ~14, 25 and 4-fold, respectively. Despite this, ACTH-treated mice did not have a depressed-phenotype ($P = 0.21$ vs vehicle). While fluoxetine produced a decrease in immobility time in chronic vehicle treated mice, this antidepressant-like effect was not observed in ACTH treated mice. In contrast, chronic ACTH treatment did not modify the responsiveness of mice to duloxetine ($P = 0.026$ vs vehicle).

Conclusions: Further evaluation of the CD-1 mouse strain demonstrated that despite resistance to fluoxetine this strain of mice was sensitive to the antidepressant-like effects of the SNRI, duloxetine. Chronic ACTH treatment also produced reduced sensitivity to fluoxetine in Balb/cJ mice, indicating a degree of resistance; however, as with the CD-1 studies, these mice remained sensitive to duloxetine. Given that duloxetine is not specifically approved for TRD, neither of the current preclinical approaches met our criteria for being predictive of efficacy in TRD. The efficiency of our partnership allowed us to reach timely predefined no-go decisions and reinforced our desire to shift towards domain-based approaches incorporating physiological endpoints to increase scope for translation. This partnership is driving efficiency through facilitating the sharing of expertise and best practices and incorporating measures of reproducibility across labs.

Keywords: treatment resistant depression, ACTH, antidepressant, preclinical model, mouse strain

Disclosure: Z. Hughes, **Part 4:** ZH is an employee of Pfizer Inc; J. Witkin, **Part 4:** JM is an employee of Eli Lilly & Co; T. Hanania, **Part 4:** TH is an employee of PsychoGenics Inc; A. Ghavami, **Part 4:** AG is an employee of PsychoGenics Inc; N. Brandon, **Part 4:** NB is a former employee of Pfizer and current employee of AstraZeneca; D. Bleakman, **Part 4:** DB is an employee of Eli Lilly & Co.; K. Rasmussen, **Part 4:** KR is an employee of Eli Lilly & Co.

M22. Witnessing Social Defeat Induces an Anxiety- and Depression-like State and Increases Nicotine Consumption

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Background: Exposure to severe stress increases the risk for developing mood and anxiety disorders, including post-traumatic stress disorder (PTSD). Recent studies in veterans with PTSD demonstrate a link between PTSD and nicotine dependence. Because of the high degree of comorbidity between mood and anxiety disorders and nicotine dependence, a hypothesis has emerged that these individuals may be smoking to manage their mood. It is also common for PTSD to develop in individuals who simply witness intense violence. However, little is known about the differences between actually experiencing and passively witnessing a traumatic event. Therefore, it is critical to develop animal models that will allow for independent assessment of the neurobiological consequences of emotional stress, including its effect on nicotine consumption.

Methods: In this study, male C57BL/6J mice were forced to witness the social defeat of another mouse. Briefly, the home cage of a male CD-1 retired breeder mouse was divided by a Plexiglas divider into two adjacent compartments. An adult male C57BL/6J mouse was introduced into the compartment territorialized by the CD-1 mouse where it was repeatedly overpowered (PS), demonstrating escape-like behaviors, vocalizations, and submissive posturing, while a second male C57BL/6J mouse witnessed (ES) this interaction from the adjacent compartment.

Results: Here we demonstrate that 10 days of ES exposure induces long-lasting deficits in a battery of behavioral assays designed to assess changes in mood. Specifically, exposure to ES and PS increases anxiety- and depression-like behaviors as measured by the elevated plus maze and forced swim test. Interestingly, mice exposed to ES and PS also displayed social avoidance in the social interaction test. ES and PS exposure also increases preference for a nicotine solution, and exposure to nicotine following stress exposure reverses the social avoidance seen in ES- and PS-exposed mice. Moreover, we observed altered gene expression within the ventral tegmental area (VTA), an area highly implicated in both responses to stress and the etiology of mood disorders, in both ES- and PS-exposed mice.

Conclusions: Taken together, these data indicate that witnessing traumatic stress is a potent stressor in mice capable of inducing lasting neurobiological alterations and subsequent nicotine treatment can normalize some of these effects.

Keywords: PTSD, nicotine, anxiety, depression, VTA

Disclosure: B. Warren, Nothing to Disclose; L. Alcantara, Nothing to Disclose; V. Vialou, Nothing to Disclose; E. Nestler, Nothing to Disclose; C. Bolanos-Guzman, Nothing to Disclose.

M23. Bacille Calmette Guérin Induces a Depressive Phenotype in 'Susceptible' Animals that is Sensitive to Antidepressants and Accompanied by Hypersensitivity to Pain Stimuli: A Preclinical Model of Comorbid Pain and Depression?

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Background: Clinical evidence suggests that pro-inflammatory cytokines play an important role in the pathology of MDD in that many patients exhibit elevated levels of circulating pro-inflammatory cytokines in the absence of medical illnesses. Thirty to 50% of cancer or hepatitis C patients treated with IFNa and/or IL-2 are susceptible to the development of psychological symptoms that culminate in a major depressive episode. Furthermore, immunotherapy to treat multiple sclerosis and stimulation of the primary host defense system precipitates depressive symptoms. Increased sensitivity to pain is a common comorbid symptom of depression and depressed patients have a higher risk of developing chronic pain. Recently, published work suggests that there is significant crosstalk between the mechanisms responsible for the comorbid relationship between pain and depression. To date, the depressive phenotype in the BCG model of chronic inflammation has not been pharmacologically characterized, nor has the model been studied for comorbid changes in pain sensitivity. The objectives of these studies were to characterize the BCG model including sensitivity to pain and to establish the pharmacological sensitivity of the BCG-induced depressive phenotype to fluoxetine, desipramine and diazepam.

Methods: To study the depressive phenotype CD-1 mice were dosed with Bacille Calmette Guerin (BCG) and measures of body weight, locomotor activity, and immobility in the tail suspension test (TST) were made. Spleen weight, plasma cytokines and lung indoleamine-2,3-dioxygenase mRNA assessments were made at experiment termination. Pharmacological studies with acute fluoxetine and desipramine were done in naïve CD-1 mice to establish doses using the TST, and in a locomotor assay to establish a non-sedating dose of diazepam. Characterization of the pharmacological sensitivity of the BCG model was done by assessing locomotor activity 6 days post BCG treatment and measuring immobility at 7 days post treatment in the presence or absence of fluoxetine (56 mg/kg), desipramine (20 mg/kg) or diazepam (1 mg/kg). To study sensitivity to pain CD-1 mice were dosed with BCG and measures of body weight taken followed by assessment of BCG-induced temporal changes in sensitivity to tactile stimuli using the von Frey assay in one experiment and assessment of BCG-induced temporal changes in sensitivity to thermal stimuli using the Hargreaves assay in a separate experiment.

Results: Ten to 30% of BCG-treated mice did not exhibit an increase in immobility and were termed 'resilient' to BCG-induced behavioral changes despite evidence of an activated immune system. BCG 'susceptible' mice exhibited increased immobility in TST and deficits in locomotor activity. The increased immobility in BCG 'susceptible' mice was attenuated by acute fluoxetine and desipramine, and exacerbated by diazepam. In a separate experiment a significant 37% increase in tactile sensitivity in BCG-treated mice was observed 3 days post BCG that was maintained out to 14 days where a 31 % increase was measured. Similarly, a significant increase in thermal sensitivity was observed in BCG-treated mice with a 44% reduction in latency to paw removal observed 1 day post BCG treatment that was maintained out to 21 days where a 42% reduction was observed.

Conclusions: We demonstrate for the first time that the depressive phenotype in this BCG model of chronic inflammation is sensitive to antidepressants and consistent with clinical reports showing that paroxetine pretreatment prior to immunotherapy can prevent the development of psychiatric symptoms. In addition, these studies provide the first evidence that the BCG model of chronic inflammation exhibits both depressive and pain phenotypes that are temporally separated from the sickness response. Therefore the BCG model will provide a novel means by which to study the cellular mechanisms responsible for comorbid depression with increased sensitivity to pain and may also serve as a means to assess novel pharmacological treatment approaches to this condition.

Keywords: Inflammation, Depression, Antidepressant, Comorbid Pain

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M24. First Hospitalization Manic Youth Show Functional Alterations during a Task of Sustained Attention

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Background: Theories of the underlying neurobiology of bipolar disorder (BD) focus on abnormalities in prefrontal-limbic emotional processing networks. BD in youth is associated with behavioral deficits and neurofunctional alterations during attention and executive function tasks. Probing these domains, including sustained attention, tests reciprocal interactions between cognitive and emotional processing networks. However, much of the research in this field is potentially confounded by the effects of repeated affective episodes, disease progression, or exposure to psychotropic medications. With these considerations in mind, we conducted a study examining the neurofunctional patterns associated with the performance of a sustained attention task in youth with BD early in their illness course. We hypothesized that bipolar youth would exhibit abnormalities in emotional processing regions and regions involved in regulating interactions between emotional and cognitive systems, including the anterior cingulate.

Methods: Adolescents ages 10-17 years 11 months with bipolar disorder type I were recruited from inpatient units during their first manic or mixed episode. Diagnosis of BD was confirmed using the Washington University in St. Louis Kiddie-Schedule for Affective Disorders and Schizophrenia (WASH-U KSADS), and all bipolar adolescents had a baseline Young Mania Rating Scale (YMRS) score ≥ 20. All participants with BD were less than 2 years from onset of their first DSM-IV-TR affective episode, had no prior psychiatric hospitalizations, and had <3 months of lifetime psychotropic medication exposure (not including stimulants). A comparison group of typically developing adolescents was also recruited. Functional magnetic resonance imaging (fMRI) was performed for all subjects during a task of sustained attention, the Continuous Performance Task-Identical Pairs version (CPT-IP). Participants were presented with a series of one-digit numbers and

asked to press a button whenever the same number appeared twice. Blocks of this active task were alternated with a control task, during which the number 1 was presented repeatedly at the same interval used in the active task. All subjects were scanned using a 4.0 Tesla (4T) Varian Unity INOVA MRI scanner. Group comparisons of demographic and performance variables were conducted using t-tests for continuous variables and chi-square tests for categorical variables. All functional imaging processing was conducted using Analysis of Functional Neuroimages (AFNI). Voxel-by-voxel comparisons of group activation were made using the AFNI program 3dRegana. Significance was defined by $p < 0.005$, with a voxel cluster size of 39. Exploratory region of interest (ROI) analysis compared activation in several relevant regions using t-tests.
Results: Thirty-eight adolescents with BD and 27 healthy adolescents participated in this study. Of the adolescents with BD, seventeen (44%) were boys, 24 (63%) were White, and the mean (SD) age 15.0 (1.5). Fourteen (52%) of the healthy adolescents were boys, 16 (59%) were White, and the mean (SD) age was 15.0 (1.5). There were no significant differences between the groups on any of these demographic variables. Discriminability, % correct responses, and reaction time were used as behavioral performance measures for the CPT-IP task. There were no significant differences between the groups in any of these measures. Voxel-by-voxel analysis revealed decreased activation in bipolar youth relative to healthy youth in several regions: bilateral thalamus, left posterior cingulate, and right anterior cingulate gyrus. There were no areas in which bipolar youth showed increased activation relative to healthy youth. Region of interest analysis revealed additional activation differences in the stratum, including the left caudate ($t = -2.22$, $p = 0.03$) and the right putamen ($t = -2.21$, $p = 0.03$), where healthy youth exhibited increased activation, while youth with BD exhibited decreased activation. A similar pattern was observed in the left ($t = -2.39$, $p = 0.02$) and right thalamus ($t = -2.13$, $p = 0.04$) in which healthy youth increased activation and youth with BD showed little task related change. ROI analysis also showed differences in the left anterior cingulate gyrus ($t = -2.66$, $p = 0.01$). In this region, both healthy and bipolar youth showed activation decreases in response to the CPT-IP task, but bipolar youth showed greater decreases than healthy controls.
Conclusions: Our results suggest that mania early in the course of BD is associated with decreased recruitment of the right anterior cingulate during the performance of a sustained attention task. ROI analysis further finds decreased recruitment of the striatum and left thalamus, as well as abnormalities in the left anterior cingulate gyrus. The presence of these abnormalities in this sample of first-hospitalization manic adolescents, who are relatively free of medication exposure, suggests that such changes are present early in the course of BD and are not due to disease progression or medication effects. These findings may represent early alterations in the interactions between cognitive and emotional processing networks. In particular, the anterior cingulate is thought to be involved in modulating the reciprocal interactions between these systems. Further research is in progress which will expand upon the sample described here and explore the effect of pharmacological treatments on the functional activation patterns described.
Keywords: bipolar disorder, adolescents, fMRI, sustained attention
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M25. Subjective Cognitive Impairment, the Pre-Mild Cognitive Impairment Stage of Eventual Alzheimer's Disease: Prospective Behavioral Markers of 2 Year Decline

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Background: There is presently widespread recognition that Alzheimer's disease (AD) begins many years prior to manifest clinical symptoms (Sperling, et al., *Alzheimers Dement*, 2011, 7:280-92). In 1982 we published the Global Deterioration Scale (GDS) which identified 2 predementia AD stages (Reisberg, et al., *Am J Psychiat*, 1982, 139:1136-39). We coined the terminology mild cognitive impairment (MCI) for GDS stage 3 (Reisberg et al., *Drug Develop Res*, 1988, 15:101-14) and demonstrated the validity of our 1986 estimate that this stage lasts $\sim$7 years prior to the advent of mild AD (Reisberg, *Geriatrics*, 1986, 41(4):30-46; Kluger et al., *J Geriatr Psych Neur*, 1999, 12: 168-79). Subsequent widespread recognition of MCI, permitted us to confirm our 1986 estimate of a mean duration of 15 years for the pre-MCI, subjective cognitive impairment (SCI, synonymous with GDS stage 2) stage of eventual AD, in a 9 year prospective study (Prichep, et al., *Neurobiol Aging*, 2006, 27:471-81; Reisberg & Gauthier, *Int Psychogeriatr*, 2008, 20:1-16). Persons with SCI have the subjective belief that their cognition and/or cognitive functioning, has declined compared with abilities 5 to 10 years previously. However, SCI persons score in the normal range on cognitive assessments. Studies indicated that SCI symptoms occur in 25 to 56% of persons in the community $\geq$ 65 years of age (see Reisberg, et al., *Alzheimers Dement*, 2010, 6: 11-24; for references). Recently, in a 7 year prospective study, we demonstrated that SCI persons have a 4.5x greater risk (hazard ratio) of declining to MCI or dementia than demographically matched older persons free of SCI with no-cognitive impairment (NCI, GDS stage 1), (Reisberg et al., *Alzheimers Dement*, 2010, 6:11-24). Herein, we report on clinical and behavioral indices of change and markers of decline in SCI persons over a 2 year period. Since AD and MCI prevention trials are likely to extend over intervals of $\sim$ 2 years, these 2 year findings should be of considerable relevance for trials of the prevention of cognitive decline in ostensibly normal persons with SCI.
Methods: Healthy subjects with SCI at baseline from our published 7 year outcome study cohort were selected at a post-baseline examination time from 1.5 to 3.0 years. One followed subject had a baseline Hamilton score $\geq$ 21, indicating significant affective symptomatology, and was therefore excluded from the analyses. The 98 subjects followed had a mean baseline age of 67.12 ± 8.8 years, 63 were female, and the mean time spent in formal education was 15. 55 ± 2.6 years. Baseline MMSE was 28.92 ± 1.23. Subjects were followed for 2.13 ± 0.30 years (range 1.57-2.93 years). Subjects were studied for: **(I)** changes on assessments over the study interval and, **(II)** the baseline differences between future decliners, defined as subjects with MCI or dementia at follow-up (F/U), and non-decliners, defined as a normal (SCI or NCI) diagnosis at F/U. The Wilcoxon Test was used for tests of difference.
Results: Part I: The GDS stage declined from 2.00 at baseline, to a mean of 2.16 at follow-up ($p < 0.01$). Of the 5 Brief Cognitive Rating Scale (BCRS), (Reisberg & Ferris, *Psychopharmacol Bull*, 1988, 24: 629-636), axes of clinical change, Axis 3, Remote Memory, declined ($p < 0.01$), as well as the BCRS axis 1-5 total scores ($p < 0.05$). No change was observed in MMSE scores. Eight psychometric tests from the WAIS (Wechsler, 1958) and the Guild

(Gilbert & Levee, *J Gerontol*, 1971, 26: 70-5) test batteries, were studied. Two tests showed declines, Paired Associate Initial Recall ($p<0.01$) and the Digit Symbol Substitution Test ($p<0.01$). One test, Designs, improved at F/U ($p<0.05$). The other 5 tests were unchanged at the F/U. **Part II:** At the 2 year F/U, 22 of the 98 subjects (22.45%) were classified as having declined clinically (i.e. they had MCI or dementia at F/U). At baseline, subjects with future decline were older and less educated ($p_s<0.05$). Future decliners had lower baseline BCRS Axis 1 (Concentration) and Axis 1-5 total scores ($p_s<0.05$), lower baseline MMSE scores ($p<0.01$), and lower baseline scores on all 8 psychometric tests ($p_s<0.05$ to <0.001).
Conclusions: On the GDS, the mean annual rate of change for the 98 subjects studied was 7.5%. This is within 1% of the anticipated change rate for a stage lasting 15 years (i.e., 6.67% change per year, with the assumptions of subjects uniformly distributed within the stage and declining at uniform mean change rates), as previously postulated and observed over much longer intervals. Hence, global changes in SCI proceeded as postulated, over this relatively brief period. Other clinical BCRS measures, and select psychometric measures, declined over the 2 year study. It is clear that for future decliners to MCI or dementia, robust clinical and psychometric decrements were already present at baseline. These baseline decrements in the cognitively normal, SCI subjects studied, were manifest and significant in future decliners, using retrospective analysis procedures. In conclusion, our observations of significant changes on the GDS, the BCRS, and select psychometric tests, over 2 years, in this study of cognitively normal, SCI subjects, with a moderate sample size, indicate that these measures have potential sensitivity as change indices in future clinical trials with the objective of preventing the development of MCI and cognitive decline.
Keywords: cognitive decline, prevention, outcome measures
Disclosure: B. Reisberg, **Part 1:** Forest Research Institute, research grant support; Medivation Inc., research grant support; MedAvante Inc., provision of research materials; Encompass Home Health Inc., provision of research materials; Equitable Life and Casualty Company, provision of research materials, **Part 2:** New York University School of Medicine, Professor of Psychiatry; Barry Reisberg, M.D., P.C., personal practice of geriatric psychiatry and provision of research materials, **Part 3:** None, **Part 4:** Forest Research Institute, research grant support; Medivation Inc., research grant support; C. Torossian, **Part 2:** New York University School of Medicine; R. Osorio, **Part 2:** New York University School of Medicine; S. Ghimire, Nothing to Disclose; K. Roy, Nothing to Disclose; P. Sharma, Nothing to Disclose; I. Monteiro, **Part 2:** New York University School of Medicine; M. Shulman, **Part 1:** Eli Lilly & Co., grant support as PI of pharmacologic investigational trial; Baxter Healthcare, grant support as sub-investigator of pharmacologic investigational trial; Pfizer, grant support as sub-investigator of pharmacologic investigational trial; Eli Lilly & Co., grant support as sub-investigator of pharmacologic investigational trial, **Part 2:** New York University School of Medicine, **Part 4:** Eli Lilly & Co., grant support as PI of pharmacologic investigational trial; Baxter Healthcare, grant support as sub-investigator of pharmacologic investigational trial; Pfizer, grant support as sub-investigator of pharmacologic investigational trial; Eli Lilly & Co., grant support as sub-investigator of pharmacologic investigational trial. All grants are awarded through New York University School of Medicine accounts. The New York University School of Medicine oversees the conduct of the trials; I. Lobach, **Part 2:** New York University School of Medicine.

M26. Increased Risk for Suicide among Schizophrenia Patients with High Premorbid IQ

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Background: Suicide is the largest cause of premature death among individuals with schizophrenia. Previous studies show that the risk for suicide among schizophrenia patients is significantly higher than that of the general population, that patients with higher IQ scores are at increased risk for suicide, and many of the suicides occurred near the onset of the illness. This study tested these findings using a large, population-based longitudinal study utilizing IQ scores assessed before the onset of illness, hence un-affected by medication, hospitalization or stigma.
Methods: Data were obtained by linking data from the Israeli military, the Israeli psychiatric hospitalization case registry, and causes of death recorded by the Israeli Ministry of Health. Subjects were 6,830 schizophrenia patients born between 1955 and 1989 who undertook mandatory, standardized IQ tests at age 17, as part of an assessment of their fitness to serve in the Israeli military, and had their first admission for a psychotic episode a year or more after the assessment. The data bases were linked in December 2006. Schizophrenia patients were then grouped into tertiles based on their premorbid IQ: $IQ<85.63$; $85.63<IQ>99.09$; and $IQ>99.09$. Hazard Ratios (HR) for suicide were calculated using Cox regression analyses adjusting for gender and year of draft board assessment.
Results: The average follow up period was 12.7 ± 9.2 years. Overall rate of suicide amongst schizophrenia patients was 2.38%, much higher than the rates of suicide in the general population (HR 83.54, 95% CI 73.22-95.31, $P<0.000$). Hazard Ratio of suicide amongst schizophrenia patients with high premorbid IQ was significantly higher compared to their lower-IQ counterparts ($HR=1.80$, 95% CI 1.37-2.37, $P<0.000$). For all premorbid IQ levels, half of all suicides occurred within the first three years after the first admission.
Conclusions: These longitudinal population-based data confirm that patients with schizophrenia have extremely high rates of suicide compared to their peers, that those with high premorbid IQ are at particularly high risk of suicide, and the frequency of suicide in schizophrenia is highest during the first years after the first admission. Patients with relatively high premorbid IQ in the initial stages of their illness should be carefully monitored for suicidal intent.
Keywords: Schizophrenia suicide, premorbid IQ, frist admission
Disclosure: M. Weiser, **Part 1:** I have recieved travel awards and/or consultanats fees and/or speakers fees and/or research grants from Jansenn, Pfizer, Lundbeck, Teva, BioLineRx, Eli Lilly, Sanofi-Aventis, Roche; O. Kapara, Nothing to Disclose; N. Werbeloff, Nothing to Disclose; E. Fruchter, Nothing to Disclose; R. Yoffe, Nothing to Disclose; M. Davidson, **Part 1:** Michael Davidson has received research grant support and/or travel support and/or speaker fees and/or consultancy fees from JNJ, Pfizer, Lundbeck, Teva, BioLineRx, Eli Lilly, Sanofi-Aventis, Roche, GSK, Envivo, Novartis, Abbott, Servier, and holds stocks in Tangent Data.

M27. Glutamate in the Associative Striatum Decreases after 4 Weeks of Antipsychotic Treatment in First-episode Psychosis: a Longitudinal 1H-MRS Study

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Background: Increased glutamate (Glu) levels in the associative-striatum have been described in patients amidst a first episode of psychosis (FEP). Whether this increase would persist after an effective antipsychotic treatment is still elusive.

Methods: The current study aimed to compare the Glu levels in the associative-striatum (dorsal-caudate, dopamine-rich region) and the cerebellar cortex (control region, negligible for dopamine) in 22 antipsychotic-naïve FEP patients and 18 healthy controls. Glu was measured using proton magnetic resonance spectroscopy (^{1}H-MRS) in a 3T scanner. Participants underwent two ^{1}H-MRS studies: patients were scanned at baseline and after 4 weeks of antipsychotic treatment, and controls were scanned at baseline and at 4 weeks after the baseline measurement. Patients were treated with oral risperidone (open-label) for 4 weeks, with dosages that were titrated based on clinical judgment. Glu concentrations were estimated using LCModel and corrected for the cerebrospinal fluid proportion within the voxel.
Results: FEP patients showed higher levels of Glu in the associative-striatum during the antipsychotic-naïve condition versus the controls ($p = 0.016$). After antipsychotic treatment, the patients showed a decrease in Glu ($p = 0.041$), whereas no differences were observed with the controls. There were no differences in cerebellar Glu between the groups at baseline or after 4 weeks.
Conclusions: Our results indicate that the increased Glu in the associative-striatum of FEP patients normalized after 4 weeks of clinically effective antipsychotic treatment. The absence of this change in the cerebellum suggests that the variation in Glu associated with psychosis is not ubiquitous throughout the brain and may be associated with dopamine-rich regions.
Keywords: 1H-MRS, Glutamate; Psychosis; Schizophrenia; Antipsychotic treatment; Associative striatum
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M28. The Promigratory Chemokine CXCL12 is Negatively Related to Neuroinflammation in the Prefrontal Cortex of People with Schizophrenia

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Background: Several lines of evidence at molecular, cellular and electrophysiological levels provide a compelling argument for deficient inhibitory interneuron function in the cortex of people with schizophrenia. Furthermore, postmortem studies also reveal subcortical alterations in neurons in the adjacent white matter, and we have recently described a link between grey matter interneuron deficit and increased density of GABA neurons in the white matter. We propose that increased white matter neuron density may represent inappropriate migration of interneurons during development, or elevated recruitment of new interneurons to the cortex in the disease. Chemokine (C-X-C motif) ligand 12 (CXCL12) is a chemoattractant that is upregulated in trauma to the brain, such as ischemia, and promotes the migration of new neurons following injury. In light of our recent findings of increased neuroinflammation in schizophrenia, we hypothesised that increased inflammatory cytokine expression would be related to increased expression of pro-migratory signals (CXCL12) in the disease.
Methods: Quantitative RT-PCR for CXCL12 mRNA was performed on cDNA from grey matter of the dorsolateral prefrontal cortex (DLPFC) of 37 people with schizophrenia/schizoaffective disorder and 37 healthy controls matched on age, brain pH, postmortem interval, RNA integrity number, gender and hemisphere. IL-6 mRNA expression and individuals in a high inflammatory group (high levels of IL-6, IL-1β, IL-8 and SERPINA3 mRNA) were identified in the same cohort previously (Fillman et al, 2012, *Molecular Psychiatry*, ePub 7th Aug).
Results: Average CXCL12 mRNA expression was unaltered in the schizophrenia group compared to controls ($t = -0.67$, $df = 71$, $p = 0.51$); however a subgroup of people with schizophrenia displaying high CXCL12 mRNA expression (>1.5SD of control mean, 5 individuals) was identified. A subgroup with low CXCL12 mRNA expression was also present in schizophrenia subjects (<1.5SD of control mean, 5 individuals). Contrary to our hypothesis more individuals with high neuroinflammation were found in the low CXCL12 expressing group (4 of the 5 individuals), while only 1/5 subjects in the high CXCL12 group had an elevated inflammatory phenotype. In controls, there was a trend for a positive relationship between CXCL12 and IL-6 mRNA expression ($r = 0.31$, $p = 0.08$); however, a negative correlation between CXCL12 and IL-6 mRNAs existed in schizophrenia ($r = -0.43$, $p = 0.01$).
Conclusions: While we predicted that individuals with schizophrenia would demonstrate high CXCL12 expression, this was only the case for a subset of subjects. Contrary to our hypothesis, elevated IL-6 expression was related to low CXCL12 expression in schizophrenia, and individuals with the lowest CXCL12 expression tended to have a high neuroinflammatory phenotype, suggesting that chemoattractive factors other than CXCL12 may influence neuronal recruitment in response to neuroinflammation in schizophrenia. While CXCL12 promotes neuronal migration to the site of injury in ischemia models, its interaction with its receptors (CXCR4 and CXCR7) also promotes tangential migration during development. Blockade of CXCR4 or CXCR7 promotes a switch from tangential to radial migration of interneurons and a reduction in CXCL12 could represent a compensatory response in an attempt to increase radial migration. Further work on chemokine and chemokine receptor alterations in white matter neurons may inform models of pathophysiology and treatments for schizophrenia.
Keywords: schizophrenia postmortem prefrontal cortex neuroinflammation chemokine
Disclosure: S. Fung, Nothing to Disclose; S. Fillman, Nothing to Disclose; C. Shannon Weickert, Nothing to Disclose.

M29. AAV Suppression of Dopamine D1 Receptors in the Striatum Impairs Probabilistic Learning via Affecting Reward-associations

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Background: Patients with schizophrenia exhibit poorer learning in social and workplace environments. Such poorer learning from positive feedback also likely deleteriously impacts the cognitive remediation/ behavioral therapy provided to these patients. Impaired reward-association learning can be quantified in a probabilistic learning task. In this task, patients with schizophrenia exhibit poor reward/stimulus association learning but normal loss/stimulus association learning. Understanding the mechanism(s) underlying such probabilistic associative learning

may aid in the development of treatments that can enhance such learning, improving work placement and augmenting cognitive remediation. There is evidence that the dopamine D1-family of receptors in the striatum is important for the acquisition of reward/stimulus associations. Evidence for the precise receptor mediating this behavior is limited however, due to non-selectivity of available agonists and antagonists and the inability of dopamine D1 knockout mice to acquire most positively rewarded tasks. We developed adeno-associated virus- (AAV) based shRNA constructs to silence *in vivo* dopamine D1 receptor expression. Thus, we examined what effect suppressing these receptors selectively in the striatum would have on probabilistic learning. By suppressing these receptors after the acquisition of holepoking for reward contingency, we determined the specific effect of suppressing this receptor on probabilistic learning. We confirmed a D1-specific effect of this shRNA-induced suppression by determining what (if any) effect the full dopamine D1-family agonist (-)doxanthrine would have on motor hyperactivity in these mice.

Methods: MaleC57BL/6N mice (n = 30) were trained to holepoke in operant chambers for a single reward (30 µl strawberry milkshake). Once at a stable level of responding, mice were matched into two groups based upon rate of responding and acquisition of the holepoking response. Either control AAV-GFP or the AAV-shRNA3 were administered bilaterally to the striatum of mice (Bregma + 0.86, M/L + −1.65, D/V −2.45 and −3.8). Mice received intermittent testing in the standard task for 40 days while the AAV-shRNA3 suppressed dopamine D1 expression. Subsequently, mice were trained in a probabilistic learning task. Two spatially divided stimuli were illuminated and a response at one stimulus (target) resulted in a reward or punishment (4 s time out) in an 80/20 ratio, while a response in the second stimulus (nontarget) resulted in a reward or punishment in a 20/80 ratio. Mice were trained in the task until target responding was > 85% for two successive days. The number of sessions to criterion and the win-stay/lose-shift strategies were compared by group. Motor activity of mice in response to (+) doxanthrine (5 mg/kg) or vehicle (saline) was assessed using the behavioral pattern monitor (BPM) in a within-subject study over 30 min. Sessions to criterion and win-stay/lose-shift strategy acquisition in the probabilistic learning task was analyzed using mixed ANOVAs, with virus as a between-subject factor, while strategy acquisition across sessions was a within-subject factor. BPM performance was analyzed using a three-way repeated measure ANOVA with virus as the between-subject factor while drug and time were within-subject factors. Significant main effects and interactions were subjected to Tukey *post hoc* analyses.

Results: Mice treated with shRNA took more sessions to achieve criterion than did GFP-treated mice ($F(1,28) = 6.6$, $p < 0.05$). A session X virus interaction trend ($F(2,56) = 2.6$, $p = 0.08$) revealed that while both groups exhibited comparable win-stay behavior on day 1, GFP-treated control mice rapidly improved over time and exhibited higher win-stay behavior in days 2 and 3 compared with shRNA-treated mice ($p < 0.05$). The virus did not affect lose-shift behavior over time however ($F < 1.6$, ns). Doxanthrine increased activity of GFP-treated mice in the first 10 min ($F(5,70) = 2.9$, $p < 0.05$), but did not affect activity of shRNA3-treated mice ($F < 1$, ns).

Conclusions: Specifically reducing dopamine D1 receptor expression in the striatum impaired probabilistic learning in mice. This impaired learning was mediated by poorer reward-associated, not punishment-associated learning. That a selective dopamine D1-family receptor full agonist increased activity in GFP- but not shRNA-treated mice supports the selectivity of learning effects to dopamine D1 receptors. These data support the premise that reduced dopamine D1 receptor expression in the striatum can deleteriously affect reward-related learning. By targeting treatment toward these receptors, such learning may be improved, perhaps facilitating work placement and cognitive remediation strategies. Future studies will determine whether (+) doxanthrine can improve probabilistic learning in mice via strengthening reward-association strategies. Such treatment studies will be conducted in animal models of schizophrenia as well as healthy control mice.

Keywords: Reward-related learning, probabilistic learning, dopamine D1 receptors, AAV, mice

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M30. Effects of Oxytocin on Social Cognition and Olfaction in Adults with Schizophrenia and Healthy Subjects

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Background: Patients with schizophrenia (SCHZ) have multiple social cognitive deficits, including difficulty in recognizing facial emotion, interpreting paralinguistic cues (e.g. sarcasm) and understanding other's mental states (i.e., theory of mind). Patients also have impaired olfaction, which is associated with worse negative symptoms and decreased social motivation. Social cognitive and olfactory deficits are correlated with worse functional outcome and quality of life and currently there are few available treatments for these deficits in SCHZ. The neuropeptide oxytocin (OT) has multiple prosocial effects when administered intranasally in humans and offers a potential remedy for these social deficits. OT has been implicated in bonding and has shown promise in enhancing social cognition in SCHZ. Further, OT signaling has been implicated in socially relevant olfaction in animals. Therefore, we investigated the effects of intranasal oxytocin on social cognition and olfaction in patients with SCHZ and healthy subjects (HS).

Methods: We administered OT (40IU) and placebo (PL) intranasally to 22 male adult patients with SCHZ and 20 HS of similar age and educational level in a randomized, double-blind, cross-over, within-subject study, with the two days of testing separated by one week. We measured performance on The Awareness of Social Inference Test (TASIT), which uses short video clips of actors to assess subjects' ability to comprehend counterfactual statements from paralinguistic cues signaling white lies (*White Lie items*), sarcasm (*Sarcasm Items*), and to make judgments about the actors' thoughts (*Theory of Mind Items*). Olfactory thresholds were measured for lyral, clove, and anise oils using a modified Munich Olfaction Test. Subjects identified the bottle with the testing compound from amongst two mineral oil containing distracter bottles in an upward step procedure with increasing geometric dilutions (12.5 X 10^{-6}% to 20% m^3/m^3). Paired t-tests were used for all comparisons and data are expressed as Mean ± (S.E).

Results: OT administration to SCHZ patients (Age: 44.0 (10.0), Education: 13.5 (2.2)) improved overall performance on TASIT (74% ± 2% vs. 70% ± 2%, $p = 0.02$), on *White Lie Items* (77% ± 2% vs. 71% ± 2%, $p = 0.02$), *Sarcasm Items* (70% ± 3% vs. 65% ± 3%, $p = 0.05$) and *Theory of Mind Items* (77% ± 2% vs. 72% ± 3%, $p = 0.05$). In HS (Age: 36.0 (13.1), Education: 15.3 (1.9)), OT administration-induced changes on these scales did not reach significance. In SCHZ patients, OT led to enhanced detection of Lyral (but not Anise or Clove) at lower concentrations

($3 \times 10^{-5}\% \pm 1 \times 10^{-7}\%$ m^3/m^3 vs. $2 \times 10^{-4}\% \pm 1 \times 10^{-7}\%$ m^3/m^3, $p = 0.03$). In HS, OT effects on olfactory thresholds did not reach significance. For TASIT, we divided subjects on a median split based on performance on the placebo day. The group of patients who scored poorly on the placebo day had greater OT-induced improvements on multiple measures (*Overall Items*: (OT-PL) $9\% \pm 2\%$ vs. $-1\% \pm 2\%$ $p = 0.001$; *Sarcasm Items*: $16\% \pm 3\%$ vs. $-4\% \pm 2\%$ $p = 0.0001$; *Theory of Mind Items*: $12\% \pm 3\%$ vs. $-3\% \pm 2\%$ $p = 0.0001$). However, in HS this relationship did not reach significance (*Overall Items*: $4\% \pm 4\%$ vs. $-2\% \pm 1\%$ $p = 0.2$; *Sarcasm Items*: $7\% \pm 5\%$ vs. $-6\% \pm 3\%$ $p = 0.06$; *Theory of Mind Items*: $5\% \pm 5\%$ vs. $-2\% \pm 2\%$ $p = 0.21$).

Conclusions: Our findings indicate that OT significantly improves SCHZ patients' ability to 1) interpret paralinguistic cues (e.g., white lies and sarcasm), and understand other's mental states (i.e., theory of mind), and 2) detect lyral at lower concentrations. The OT-induced improvement in social cognition is clinically significant because deficits in these domains are strong predictors of functional outcome in SCHZ and are currently difficult to treat. The OT-induced improvement in detection of lyral demonstrates that OT may be the first pharmacological agent to remediate the olfactory deficits in SCHZ. Furthermore, the selectivity of this effect for lyral fits with previous data indicating that patients with SCHZ are selectively impaired at detecting lyral possibly due to cAMP signaling dysfunction and that OT can increase cAMP signaling *in vitro*. Finally, it appears that OT has differential effects depending on an individual's baseline ability in that subjects who score poorly on the placebo day have large significant improvements on performance when administered OT. The underlying mechanisms for this differential effect remain unknown, however, OT may be improving basic, early sensory processing, such as gaze to the eye region, which helps individuals with poor baseline social cognition. In sum, our data provide support for using OT as a pharmacological agent to remediate multiple social deficits in SCHZ. Larger studies focused on patients with SCHZ who have significant baseline deficits in social cognition are needed to confirm and extend our findings.

Keywords: schizophrenia, oxytocin, social cognition, olfaction, pharmacology

Disclosure: J. Woolley, Nothing to Disclose; B. Chuang, Nothing to Disclose; O. Lam, Nothing to Disclose; K. Rankin, Nothing to Disclose; D. Mathalon, Nothing to Disclose; S. Vinogradov, Nothing to Disclose.

M31. Neuroanatomical Correlates of Apathy in Late Life Depression and Antidepressant Treatment Response

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Background: The symptom of apathy is associated with a number of poor clinical outcomes and is a salient feature of geriatric depression, yet little is known about the neurobiology and treatability of apathy. This study examined the incidence of apathy in late life depression, the response of apathy to SSRI treatment, and the neuroanatomical correlates of apathy responsiveness to SSRI treatment.

Methods: Participants were 45 non-demented elderly individuals with non-psychotic major depression and 43 elderly comparison subjects. After a 2-week single-blind placebo period, subjects who still had a Hamilton Depression Rating Scale (HDRS) $\geq$ 18 received escitalopram 10 mg daily for 12 weeks. Apathy and depression were quantified with the Apathy Evaluation Scale (AES) and HDRS, respectively, at baseline and again following 12 weeks of treatment. MRI scans were acquired on a 1.5 Tesla scanner at baseline and data were collected for concurrent structural and diffusion tensor imaging examination of anterior cingulate gray matter and associated white matter tracts. Gray matter volumes of anterior cingulate cortex (ACC) subregions were calculated from manual outlines of regions of interest (dorsal, rostral, anterior subgenual, and posterior subgenual). For white matter analysis, fractional anisotropy (FA) was determined in specific regions using the Reproducible Object Quantification Scheme (ROQS) software that operates on non-normalized data.

Results: At baseline, 35.5% of depressed patients met criteria for clinically significant apathy AES $\geq$ 36.5). The incidence of apathy in this group declined to 15.6% ($p < 0.1$), following treatment with escitalopram, while 43% of those initially suffering from apathy continued to meet criteria for clinically significant apathy. The degree of improvement in apathy following escitalopram treatment was independent of change in HDRS scores but was correlated with specific neuroanatomical measures: larger left posterior subgenual anterior cingulate cortical volumes (psgACC) and higher fractional anisotropy (FA) of the left uncinate fasciculus at baseline.

Conclusions: While apathy is a symptom that is prevalent in geriatric depression, it may also be separable from depression with regards to its responsiveness to specific medication treatment and its underlying neuroanatomical correlates. Structural abnormalities of the left posterior subgenual cingulate and left uncinate fasciculus may impede adequate treatment of apathy by interfering with prefrontal cortical recruitment of limbic region activity relevant to the production of motivated behavior.

Keywords: apathy, late-life, depression, neuroimaging, geriatric

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M32. Central Oxytocin in Social and Cued Fear Conditioning in Mice: Specific Role of the Dorsolateral Septum

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Background: The nonapeptide oxytocin (OXT), which is synthesized in the paraventricular and supraoptic nuclei of the hypothalamus, has received substantial interest in recent years due to its pro-social and anxiolytic properties. OXT has also been shown to attenuate the behavioral and physiological responses to stressors in both rodents and humans. Accordingly, it has been recently proposed as a potential therapeutic agent for disorders associated with social deficits, such as social anxiety disorder (SAD) or autism spectrum disorder (ASD). Using mouse models of social fear conditioning (1) and cued fear conditioning (2) we investigated whether central OXT administration facilitates the extinction of social and cued fear, respectively.

Methods: For social fear conditioning, mice received foot-shocks when they approached and investigated a con-specific. Social fear was expressed as decreased social investigation and aversive responses towards the social stimuli (freezing, stretched approaches). For cued fear conditioning, animals were exposed to tones that co-terminated with foot-shocks; cued fear was expressed as increased freezing responses to the tone. In both paradigms, unconditioned control animals were exposed to the respective conditioning stimuli in the absence of the foot-shock. Social and cued fear extinction were performed 24 h later in a novel environment. OXT was administered into the lateral ventricle (icv) 10 min before the extinction procedure.

Results: Central administration of OXT, but not of the closely related vasopressin, extinguished social fear without decreasing general anxiety. In contrast, central OXT impaired cued fear extinction, without increasing fear expression itself. Both the facilitatory effect on social fear extinction and the impairing effect on cued fear extinction were mediated by OXT receptors (OXTR),

as icv administration of an OXTR antagonist prior to OXT blocked the observed effects. Furthermore, blockade of OXT neurotransmission in unconditioned animals impaired social investigation during social fear extinction, confirming the important role of endogenous OXT for naturally-occurring social approach behavior [3]. We further assessed the consequences of social fear conditioning on the brain OXT system. Social fear was accompanied by an increased OXTR binding in the dorsolateral septum (DLS), right central amygdala, dentate gyrus and cornu ammunis, which normalized after extinction of social fear. These regions are likely to be part of a brain network involved in the development of social fear. Infusion of OXT into the DLS extinguished social fear in a similar way as seen after icv infusion, indicating a prominent role of OXT within this region in social fear.
Conclusions: These results imply that OXT exerts differential effects on fear extinction in social versus non-social contexts. Specifically, they suggest OXT as potential add-on drug in the treatment of disorders associated with social fear, such as SAD and ASD. However, in patients where the fear does not involve a social component, such as post-traumatic stress disorder, OXT may even delay fear extinction. 1 Toth I, Neumann ID, Slattery DA (2012) Social fear conditioning: a novel and specific animal model to study social anxiety disorder. Neuropsychopharmacology 37:1433-43 2 Toth I, Neumann ID, Slattery DA (2012) Central administration of oxytocin receptor ligands affects cued fear extinction in rats and mice in a timepoint-dependent manner. Psychopharmacol (Berl) 3 Lukas M, Toth I, Reber SO, Slattery DA, Veenema AH, Neumann ID (2011) The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice. Neuropsychopharmacology 36:2159-68.
Keywords: oxytocin, social fear, fear conditioning, oxytocin receptors, mice
Disclosure: I. Toth, Nothing to Disclose; D. Slattery, Nothing to Disclose; I. Neumann, Nothing to Disclose.

M33. Brain-Derived Neurotrophic Factor, Interleukin-6, and Salivary Cortisol Levels in Patients with Major Depressive Disorder Treated with Desvenlafaxine vs Placebo

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Background: Brain-derived neurotrophic factor (BDNF), interleukin (IL)-6, and salivary cortisol are potential biomarkers for depression or treatment response in patients with major depressive disorder (MDD). The objective of this analysis was to assess relationships between these 3 biomarkers and depression severity and treatment response in patients enrolled in a double-blind, placebo-controlled trial of desvenlafaxine 50 mg/d for MDD.
Methods: Employed outpatients with MDD were randomly assigned to 12 weeks of double-blind treatment with desvenlafaxine 50 mg/d or placebo (2:1). Baseline severity was assessed using the 17-item Hamilton Rating Scale for Depression ($HAM\text{-}D_{17}$); treatment response at week 12 (last observation carried forward [LOCF]) was based on $HAM\text{-}D_{17}$ total score and response ($\geq 50\%$ decrease from baseline in $HAM\text{-}D_{17}$ total score) and remission ($HAM\text{-}D_{17}$ total score ≤ 7) status. Saliva (cortisol) and blood (BDNF, IL-6) samples for biomarker assay were collected at baseline and week 12 or at early discontinuation. Spearman correlations were calculated between biomarkers at baseline, and between biomarkers and $HAM\text{-}D_{17}$ total score at baseline. Logistic regression analyses were used to assess the predictive value of baseline biomarkers for treatment response at week 12, or effect of baseline disease severity on biomarker change at week 12, while adjusting for baseline biomarker level or baseline $HAM\text{-}D_{17}$ total score, treatment, and geographic region.
Results: A total of 437 patients were randomly assigned to treatment; 427 patients who received ≥ 1 dose of study drug and had baseline and ≥ 1 on-therapy primary efficacy evaluations were included in the analysis. At baseline, there was a statistically significant positive correlation between levels of IL-6 and BDNF (Spearman correlation coefficient [r_s] = 0.120; $P = 0.014$), but no significant correlation between biomarker levels and baseline $HAM\text{-}D_{17}$ total score (absolute value of all r_s, ≤ 0.061). Desvenlafaxine 50 mg/d treatment significantly reduced $HAM\text{-}D_{17}$ total score from baseline at week 12 (LOCF) compared with placebo (adjusted mean difference [95% confidence interval], 2.12 [0.78, 3.46]; $P = 0.002$), but no treatment effects were observed for any of the 3 biomarkers. No significant correlations were observed between the change from baseline in any biomarker level and change in $HAM\text{-}D_{17}$ total score at week 12 (LOCF), either overall, or in the desvenlafaxine or the placebo treatment group (absolute value of all r_s, 0.003–0.196). Logistic regression models indicated that baseline levels of BDNF, IL-6, and salivary cortisol did not significantly predict response to treatment based on $HAM\text{-}D_{17}$ total score or response or remission status at week 12 (LOCF). Although median increase in BDNF was not significantly different between desvenlafaxine (13.7%) and placebo (5.7%) groups, the increase was significantly greater ($P = 0.003$) in patients with more severe depression at baseline, representing a 33.4% median increase when $HAM\text{-}D_{17} > 22$ vs 4.3% when $HAM\text{-}D_{17} \leq 22$. No similar findings were observed for IL-6 or salivary cortisol.
Conclusions: In this study, the first of its kind in which BDNF, IL-6, and cortisol were all measured in a single trial, weak or no relationships were observed at baseline between biomarkers or between biomarkers and disease severity. While baseline biomarker level did not predict treatment response, improvement in BDNF was significantly greater among patients who were more depressed at baseline.
Keywords: depression; biomarker; BDNF; cortisol; Desvenlafaxine
Disclosure: P. Ninan, **Part 3:** Former employee of Pfizer Inc.; R. Shelton, **Part 1:** Consultant: Eli Lilly and Company; Cyberonics, Inc.; Janssen Pharmaceutica; Medtronic, Inc.; Pamlab, Inc.; Pfizer, Inc.; Ridge Diagnostics; Takeda Pharmaceuticals, **Part 4:** Grant/research support: Bristol-Myers Squibb; Eli Lilly and Company; Elan, Corp.; Euthymics Bioscience; Forest Pharmaceuticals; Janssen Pharmaceutical Novartis Pharmaceuticals Otsuka Pharmaceuticals; Pamlab, Inc.; Pfizer, Inc.; Repligen, Corp.; Ridge Diagnostics; St. Jude Medical, Inc.; Takeda Pharmaceuticals; W. Bao, **Part 3:** Current employee of Pfizer Inc; C. Guico-Pabia, **Part 3:** Current employee of Pfizer Inc.

M34. Baseline Working Memory Abnormalities in Current Major Depression as Detected by Magnetoencephalography

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Background: Working memory difficulties are observed during depressive episodes in both major depressive disorder (MDD) [Marazziti *et al.* (2010) *Eur J Pharmacol* **626**(1): 83-6] and bipolar disorder (BD) [Solé *et al.* (2011) *Psychol Med* **41**(9): 1791-803]. Functional magnetic resonance imaging (fMRI) studies in MDD and BD have revealed altered blood-oxygen dependent signals (BOLD) in brain regions including prefrontal cortex and anterior cingulate cortex using a matching N-back task [Harvey *et al.* (2005) *Neuroimage* **26**(3): 860-9, Matsuo *et al.* (2007) *Mol Psychiatry* **12**(2): 158-66, Townsend *et al.* (2010) *Psychiatry Res* **182**(1): 22-9]. Magnetoencephalography (MEG) provides greater temporal resolution than fMRI, allowing the investigation of features of neural activity that are not detectable by fMRI. In the current study, we evaluated MEG beta-band power during

performance of a working memory task in healthy participants and patients with MDD and BD.
Methods: Currently depressed, unmedicated patients with MDD (n = 34; MADRS 33.2 ± 5.4) and BD treated with only lithium or valproate (n = 18; MADRS 33.4 ± 6.2) and 25 well-matched healthy controls (HCs) were scanned using MEG while they performed a modified N-back task with 3 difficulty levels (0-back, 1-back, 2-back) requiring recall but not matching. Accuracy and reaction time on all trials free of MEG artifacts were compared between groups using a repeated-measures ANOVA. Scans were acquired on a 275 sensor whole-head CTF system at 1200 Hz with 0-300 Hz bandwidth and 3rd gradient balancing. T1-weighted MRI scans were acquired for coregistration and source localization, and were preprocessed using Analysis of Functional Neuroimages (AFNI) software. A Nolte realistic head model was used for the forward solution, and source localization was performed using synthetic aperture magnetometry (SAM) for data in the beta band (14-30 Hz). A data window of −0.75 s to 0.5 s around each response was used to calculate beamformer weights, and this window was increased to −0.75 s to + 0.75 s around the responses if the shorter window produced artifactual results. Power was calculated using a time window of −0.25 s to 0.25 s. Mann-Whitney U maps were created of power differences between the 2 vs. 1-back, 2 vs. 0-back, and 1 vs. 0-back tasks for each subject. For group analysis, standard t-tests were performed to compare the combined patient group, and each patient group alone to HCs, using age and gender as covariates. A false discovery rate (FDR) correction for multiple comparisons was applied over the whole brain.
Results: The groups did not significantly differ ($F = 1.154$, $p = 0.321$) on task accuracy. However, accuracy decreased with increasing difficulty (Wilks' Lambda; $F = 28.957$, $p < 0.001$). MDD, BD, and HC groups significantly differed on reaction time ($F = 4.381$, $p = 0.016$), with BD subjects performing significantly slower ($p = 0.005$) and MDD subjects showing a trend towards slower performance ($p = 0.052$) as compared to HCs. HCs exhibited reduced beta-band power between the 2 vs. 1-back conditions, indicating more beta desynchronization and, concomitantly, increased activation while performing the more difficult condition. Depressed patients exhibited greater beta band power with increasing demand than the HCs (indicating blunted activation) in the following brain regions (all implicated in various elements of working memory): inferior, middle, and superior temporal gyri, posterior cingulate, caudate, superior frontal gyrus/frontal polar cortex, medial frontal gyrus/dorsolateral prefrontal cortex (dlPFC) and cuneus/precuneus. On subgroup analysis, the BD group differed significantly from HCs in the right superior temporal gyrus, posterior cingulate, and left dlPFC. Subgroup analysis of the MDD patients showed trend level ($q < 0.059$, minimum q) differences in the left temporal cortex, left dlPFC, left insula, and cuneus.
Conclusions: We observed increased beta-band power (less beta desynchronization/less activation) in the 2 vs. 1-back tasks in brain regions implicated in working memory in active major depression regardless of diagnosis as compared to controls. Blunted increases in activation in depressed subjects in the 2 vs. 1-back tasks may signify a "ceiling effect" *i.e.,* depressed patients maximally recruit salient working memory circuitry during the performance of less difficult (*i.e.* 1-back) tasks as compared to HCs who recruit only at greater cognitive demand (*i.e.* 2-back).
Keywords: Working Memory, Depressive Episode, Major Depressive Disorder, Bipolar Disorder, Magnetoencephalography
Disclosure: M. Niciu, Nothing to Disclose; A. Nugent, Nothing to Disclose; C. Marquardt, Nothing to Disclose; T. Holroyd, Nothing to Disclose; G. Salvadore, **Part 1:** Dr. Salvadore is a full-time employee of Janssen Pharmaceuticals, LLC.; M. Furey, **Part 4:** Dr. Furey is listed as a co-inventor on a patent application for the use of scopoloamine in major depression. Dr. Furey has assigned her rights in the patent to the U.S. government but will share a percentage of any royalties that may be received by the government; C. Zarate, **Part 4:** Dr. Zarate is listed as a co-inventor on a patent application for the use of ketamine and its metabolites in major depression. Dr. Zarate has assigned his rights in the patent to the U.S. government but will share a percentage of any royalties that may be received by the government.

M35. Detecting Activity-evoked pH Changes in Human Brain

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Background: While pH changes have been suggested to occur with brain function, a limited number of tools exist to measure pH dynamics with sufficient temporal and spatial resolution. Therefore, we investigated the ability to detect brain pH changes with magnetic resonance imaging using T1 relaxation in the rotating frame (T1rho).
Methods: The pH sensitivity of T1rho was tested in phantoms, mice, and humans in response to manipulating systemic carbon dioxide (CO_2) levels and during activation of the visual cortex with a flashing checkerboard task.
Results: In phantoms, T1rho was linearly sensitive to pH in the physiological range. In mice, T1rho correlated closely with direct pH measurements simultaneously obtained with a fiberoptic pH sensor. In humans, T1rho was sensitive to pH changes induced by manipulating end-tidal CO_2. Moreover, T1rho, detected pH changes during functional activation of the visual cortex with a flashing checkerboard task. ^{31}P-spectrocopy at the same site also identified a localized acidosis. The activity-evoked response detected by T1rho in the visual cortex correlated closely with blood oxygenation level-dependent contrast changes. However, T1rho was not directly sensitive to blood oxygen content.
Conclusions: This study suggests that a local acidosis occurs with brain function that can be potentially measured using MRI techniques. It also suggests that T1rho imaging can serve as a novel functional imaging technique that is able to access pH dynamics *in vivo*. A number of psychiatric disorders may have abnormalities in pH regulation and this imaging technique may provide novel insight onto the neurobiology of these disorders.
Keywords: functional brain imaging pH T1rho
Disclosure: J. Wemmie, Nothing to Disclose; V. Magnotta, Nothing to Disclose.

M36. Structure-Functional Selectivity Relationship Studies of Beta-arrestin-biased Dopamine D2 Receptor Agonists

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Background: G protein-coupled receptors (GPCRs) signal not only via canonical pathways involving heterotrimeric large G proteins, but also via non-canonical G protein-independent interactions with other signaling proteins including, most prominently, β-arrestins. The process by which GPCR ligands differentially modulate canonical and non-canonical signal transduction pathways is a phenomenon known as "functional selectivity". Clearly, functionally selective ligands are extremely useful for elucidating the key signal transduction pathways essential for both the therapeutic actions and side-effects of drugs. However, very few such ligands have been created and very little purposeful attention has been devoted to studying what we term: 'structure-functional selectivity relationships' (SFSR). In this presentation, we report comprehensive SFSR studies focused on exploring four regions of the aripiprazole scaffold, which resulted in the discovery of extremely β-arrestin-biased agonists of dopamine D_2 receptors

(D_2R). We also describe antipsychotic drug-like activities of these extremely β-arrestin-biased D_2R agonists in mice and their drug-like properties including selectivity and pharmacokinetic (PK) parameters.
Methods: Novel analogs of aripiprazole were designed and synthesized. These newly synthesized compounds were evaluated in D_2R binding, D_2R-mediated cAMP accumulation, and D_2R-mediated β-arrestin-2 translocation Tango assays to assess their functional selectivity profiles. Extremely β-arrestin-biased D_2R agonists were tested in C57BL/6 wild-type (WT) and β-arrestin-2 knockout (β-ARR2 KO) mice for their ability to inhibit phencyclidine (PCP)-stimulated hyperlocomotion. A broad range of GPCR binding and functional assays were used to assess selectivity. Standard PK studies were conducted to assess PK parameters of β-arrestin-biased D_2R agonists in mice.
Results: To determine whether modifying various structural motifs of the aripiprazole scaffold could result in biased compounds that favor either cAMP or β-arrestin signaling, we intensively investigated the following four regions of the aripiprazole template: (1) the left-hand side (LHS) phenyl ring with various substitution; (2) the middle cyclic amino moiety; (3) the central linker; and (4) the right-hand side (RHS) bicyclic aromatic moiety. In total, we prepared > 150 novel compounds. Evaluation of these compounds in D_2R binding, G_i-mediated cAMP accumulation, and β-arrestin-2 translocation Tango assays revealed the following SFSR trends: (1) the electronic nature (e.g., electron donating or withdrawing), steric bulkiness, and substitution pattern of substituents on the LHS phenyl ring did not, apparently, have significant effects on patterns of D_2R functional selectivity; (2) the homopiperazine group as a middle amino moiety reduced efficacy for activating both β-arrestin and G_i pathways; (3) several conformationally-constrained central linkers could lead to significant bias for D_2R-mediated β-arrestin-2 over G_i activities. However, these central linkers resulted in significant losses of potency; and (4) a number of RHS aromatic groups such as benzothiazole, indazole, and benzimidazolone led to significant bias for D_2R-mediated β-arrestin-2 over G_i activities. Importantly, we observed that subtle structural changes could result in substantial changes in functional selectivity. Through these comprehensive SFSR studies, we discovered: (1) extremely β-arrestin-biased D_2R agonists UNC9994 and UNC9995; and (2) high potency and low efficacy β-arrestin-biased D_2R agonists UNC9975 and UNC0006. These compounds were simultaneously full or partial agonists for D_2R/β-arrestin-2 interactions and inactive at G_i-regulated cAMP production. Importantly, these β-arrestin-biased D_2R agonists markedly inhibited PCP-induced hyperlocomotion in WT mice and the significant antipsychotic drug-like activities were either completely abolished or significantly attenuated in β-ARR2 KO mice. In addition, these β-arrestin-biased D_2R agonists had similar GPCR selectivity as aripiprazole and excellent mouse PK parameters including high CNS penetration.
Conclusions: We designed and synthesized a series of novel compounds for exploring four regions of the aripiprazole scaffold. Comprehensive evaluation of these compounds in D_2R binding and functional assays revealed a number of important SFSR findings. Combining the best structural motifs identified from these studies into single molecules resulted in the discovery of extremely β-arrestin-biased D_2R agonists UNC9994 and UNC9995 and high potency and low efficacy β-arrestin-biased D_2R agonists UNC9975 and UNC0006. Findings from our *in vivo* studies of these β-arrestin-biased D_2R agonists in wild-type and β-arrestin-2 knockout mice suggest that β-arrestin recruitment and signaling can be a significant contributor to antipsychotic efficacy. Our combined medicinal chemistry and pharmacological profiling approach provides the biomedical community a successful proof-of-concept for how functionally selective ligands can be discovered.

Keywords: Functional selectivity, beta-arrestin-biased ligands, D2 receptors, structure-functional selectivity relationship, antipsychotics
Disclosure: J. Jin, Nothing to Disclose; X. Chen, Nothing to Disclose; M. Sassano, Nothing to Disclose; V. Setola, Nothing to Disclose; M. Chen, Nothing to Disclose; W. Wetsel, Nothing to Disclose; B. Roth, Nothing to Disclose.

M37. Safety and Efficacy of Olanzapine/Fluoxetine Combination Versus Placebo in Patients Aged 10 to 17 in the Acute Treatment of Major Depressive Episodes Associated with Bipolar I Disorder

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Background: Olanzapine/fluoxetine combination (OFC) is approved for the acute treatment of depressive episodes associated with bipolar I disorder in adults. This study assessed the safety and efficacy of OFC for the treatment of major depressive episodes associated with bipolar I disorder in children and adolescents.
Methods: Inpatients or outpatients – 10 to 17 years of age meeting DSM-IV-TR criteria for bipolar I disorder, current episode depressed as confirmed by the Kiddie-Schedule for Affective Disorders-Present and Lifetime, with baseline Children's Depression Rating Scale-Revised (CDRS-R) total score ≥40, Young Mania Rating Scale (YMRS) total score ≤15, and YMRS item 1 ≤2 - were randomized to OFC (n = 194) or placebo (n = 97) for up to 8 weeks of double-blind treatment. Dosing was initiated at 3 mg olanzapine/25 mg fluoxetine (3/25) per day and titrated up to 12/50 by the end of week 2, with flexible dosing thereafter (6/25, 6/50, 12/25 or 12/50). The primary efficacy measure was mean change from baseline to week 8 in CDRS-R total score using mixed-model repeated measures methodology. Time to response and remission were assessed using Kaplan-Meier methodology. Response was defined as a 50% reduction in CDRS-R from baseline with YMRS item 1 ≤2. Remission was defined as a CDRS-R total ≤28 with YMRS total ≤8 and Clinical Global Impression (CGI)-Severity of Illness-Bipolar Version score ≤3. Data from 2 of 41 sites were excluded due to data integrity failure; final analyses were based on 170 OFC patients and 85 placebo patients.
Results: Mean baseline CDRS-R score was 54.6 (SD 10.0) for the OFC group and 53.7 (SD 8.1) for the placebo group. Least squares mean change at week 8 was −28.4 (SE 1.1) for OFC and −23.4 (SE 1.5) for placebo, indicating significant improvement in both groups but superior improvement in the OFC group (p = .003). OFC patients showed statistically significantly greater improvement relative to placebo at week 1 (p = .02) and throughout the remaining 8 weeks (p values <.01). A total of 78% of OFC patients compared to 59% of placebo (p = .003) achieved response, with the OFC group demonstrating a significantly shorter time to response than placebo (p = .001). A total of 59% of the OFC group vs 43% of placebo achieved remission (p = .035), with the OFC group experiencing a significantly shorter time to remission than placebo (p = .028). There was no significant between-group difference in treatment-emergent mania (OFC 1%, placebo 0%, p = 1.0).
A total of 68% of the OFC group vs 71% of the placebo group completed the study. Most common reason for discontinuation was adverse event (14% OFC, 6% placebo, p = .06). Seventy-four percent of the OFC group and 58% of placebo (p = .015) experienced treatment-emergent adverse events, with weight gain, appetite increase, and somnolence the most frequent for OFC that were greater than in placebo. Mean weight gain at endpoint was significantly greater in the OFC group (4.4 kg) than in placebo (0.5 kg, p <.001). Weight increases ≥7% of baseline occurred in 52% of the OFC group vs 4% of placebo (p <.001). Compared with placebo, OFC-treated patients had statistically significantly higher rates of treatment-emergent abnormally high levels of alanine

aminotransferase (46% vs 3%), aspartate aminotransferase (34% vs 8%), and fasting total cholesterol (29% vs 8%), LDL cholesterol (20% vs 7%), and triglycerides (52% vs 27%). Greater mean increases in prolactin (8.7 ng/mL vs 0.7, $p < .001$), heart rate (4.5 vs 1.0 bpm, $p = .013$) and QTc interval (8.2 vs −1.1 msec, $p < .001$) were also noted for the OFC group compared to placebo. Twelve percent of the OFC group experienced a change in QTcF of ≥30 msec compared to 1% of placebo ($p = .005$), but no patients developed QTcF ≥480 msec anytime. Rates of extrapyramidal symptoms were low (≤1%) and not statistically different between groups. Rates of suicide attempts were also low (1 patient in each group).
Conclusions: OFC demonstrated efficacy for the acute treatment of a major depressive episode in children and adolescents (ages 10 to 17) with bipolar disorder. Safety findings were generally consistent with those historically observed in adults treated with OFC or adolescents treated with olanzapine monotherapy, although a modestly higher increase in QTc was also observed. OFC is not approved for patients <18 years of age.
Keywords: Olanzapine/fluoxetine, bipolar I disorder, safety, efficacy
Disclosure: M. DelBello, **Part 1:** Lecture Burear: Bristol-Myers Squibb and Merck, **Part 3:** Consulting/Advisory Board/Honoraria: Merck; Schering-Plough; and Pfizer, **Part 4:** Research Support from: AstraZeneca; Eli Lilly and Company; Johnson and Johnson; Janssen; Pfizer; Otsuka; Sumitomo; NIDA; NIMH; NIAAA; NARSAD; GlaxoSmithKline; Merck; Novartis; and Lundbeck; H. Detke, **Part 1:** Full-time employee and minor shareholder of Eli Lilly and Company; J. Landry, **Part 1:** Full-time employee of Eli Lilly Canada, Inc.; R. Usher, **Part 1:** Retired employee of Eli Lilly and Company; R. Schroer, **Part 1:** Full-time employee of Eli Lilly and Company; M. Dingankar, **Part 1:** Full-time employee and minor shareholder of Lilly Research Centre at Erl Wood, United Kingdom.

M38. Basolateral Amygdala Stimulation Produces Heterosynaptic Suppression of Inputs from Other Temporal Cortical Structures in the Prefrontal Cortex

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Background: The prefrontal cortex (PFC) is critical for executive control, behavioral flexibility, and working memory. These functions are dependent on highly interconnected circuits that include limbic substrates, such as the amygdala and hippocampus, as well as primary and associative cortices. Afferents arriving from the amygdala likely provide information related to emotional states and may adjust PFC function. It is conceivable that strong amygdala activation reduces PFC synaptic responses to other afferents in order to allow an override of other influences in cases of strong emotional activation. Here, we examined the interactions between the basolateral amygdala (BLA) and other temporal cortical nuclei including the ventral hippocampus (VH) and the amygdalopiriform transition area (APiri), an associative cortical structure, on individual PFC pyramidal neuron physiology using *in-vivo* intracellular recordings.
Methods: Using sharp microelectrodes, medial PFC (mPFC) neurons were recorded intracellularly in anesthetized rats, and synaptic responses were generated by electrical stimulation of the BLA and fimbria (the fiber carrying hippocampal inputs to the PFC) or APiri. To determine whether BLA train stimulation attenuated fimbria- or APiri-evoked responses in the mPFC, a baseline test pulse (S1) was applied to the fimbria or APiri followed by conditioning single pulse or burst stimulation of the BLA (10 pulses; 10, 20, or 50 Hz) 500 ms later. A test synaptic response (S2) was evoked at varying delays (50, 100, 150, 300, or 500 ms) after the last BLA pulse in the train. In a separate group of rats, viral-mediated expression of channelrhodopsin-2 (ChR2) in hippocampal projection neurons was utilized to determine whether BLA burst stimulation also suppressed optically-evoked fimbria responses in the PFC.
Results: BLA burst stimulation attenuated fimbria- and APiri-S2-evoked EPSP amplitudes in a time- and frequency-dependent manner, with S2 responses at short delays being inhibited more robustly than those at longer delays. These effects were also observed using optical stimulation of the fimbria in rats expressing a ChR2 in ventral hippocampal neurons. This interaction is activity-dependent as single pulse BLA stimulation is without effect on fimbria- or APiri-evoked S2 responses at all delays. Fimbria and APiri train stimulation did not alter BLA-evoked responses, suggesting that heterosynaptic suppression of temporal cortical inputs is unidirectional and only produced by activation of the BLA-mPFC pathway.
Conclusions: A BLA-evoked heterosynaptic suppression of other temporal cortical inputs could be critical for the selection of the appropriate behavioral response to fear-inducing or conditioned stimuli that would activate the amygdala. The mechanisms involved in this interaction remain to be discovered.
Keywords: Prefrontal cortex, Amygdala, Hippocampus, Electrophysiology, Optogenetics
Disclosure: H. Tejeda, Nothing to Disclose; P. O'Donnell, **Part 1:** Consultant for Roche Pharmaceuticals.

M39. A 0.23 Mb Region on Mouse Chromosome 11 Contains Three Possible Quantitative Trait Genes Influencing Methamphetamine Sensitivity

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Background: Sensitivity to the locomotor stimulant properties of drugs of abuse is mediated by shared neurocircuitry with drug reward; thus, determining its genetic basis will enhance our understanding the neurobiology of addiction. We previously used mouse lines derived from C57BL/6J (B6) and DBA/2J (D2) strains that were selected for high and low sensitivity to methamphetamine (MA)-induced locomotor activity and identified a quantitative trait locus (QTL) on chromosome 11. In the present study, we phenotyped a genome-wide B6 x D2 F_2 cross and an F_8 advanced intercross to replicate this QTL and fine map the locus. To further aid in identifying the quantitative trait gene(s) (QTGs) responsible for the QTL, we generated over 15 subcongenic and sub-subcongenic lines carrying D2-derived intervals on a B6 background (B6.D2) spanning the entire chromosome 11.
Methods: 676 F_2 mice and 552 F_8 mice were administered saline injections (10 ml/kg) on Days 1 and 2 in the open field (37.5 cm x 37.5 cm; AccuScan Instruments). On Day 3, mice were injected with MA (2 mg/kg, i.p.) and the total distance traveled was recorded over 30 min. Three B6.D2 congenic lines containing large D2-derived portions of chromosome 11 were originally obtained from Dr. Aldons (Jake) Lusis's laboratory at UCLA and have since been backcrossed to B6 to introduce new recombination events and generate new lines. These subcongenic and sub-subcongenic lines were tested for MA sensitivity in an identical manner as described above and were always tested alongside wildtype littermate control mice. Congenic mice were genotyped using custom-made fluorescent markers from Applied Biosystems or PCR and traditional Sanger sequencing of genomic regions capturing B6/D2 SNPs. SNPs were chosen from the Mouse Phenome Database and the Sanger Institute SNP query website.
Results: Genome-wide analysis of the F_2 cross, but not the F_8 cross, revealed a large, time-dependent QTL on chromosome 11 for MA-induced locomotor activity on Day 3 (peak LOD = 10) with a 1.5 LOD support interval of 30-70 Mb. The results of a congenic line

(0-80 Mb) confirmed this QTL. Additionally, subcongenic lines spanning the region uncovered multiple, smaller-effect QTLs as evidenced by their different time-dependent effects on the time course for MA-induced locomotor activity and their different modes of inheritance. One particular subcongenic line (50-68 Mb) that flanked the peak marker of the F_2 study (55 Mb) captured a QTL that was confirmed via re-analysis of F_8 markers within this region. The latter observation indicated that the F_8 study was likely underpowered to reach significance for detecting this smaller-effect QTL. Next, we further dissected the 50-68 Mb locus by generating two sub-subcongenic lines that were congenic for a region spanning approximately 50-60 Mb. Owing to a fortuitous recombination event, these two lines possessed genotypes that differed only within a 0.23 Mb genomic region (50,186,508 Mb - 50,418,318 Mb). The inheritance of the B6 allele within this small region was sufficient to reverse the phenotype from D2 to B6, demonstrating that at least one allele within this interval contributes to the QTL. There are only 3 annotated genes within the interval (*Hnrnph1*, *Rufy1*, and *Adamts2*). Thus, QTG identification is a tractable goal.
Conclusions: Using a combined approach of F_2, F_8 advanced intercross, and congenic analysis, we identified a 0.23 Mb region on chromosome 11 influencing MA-induced locomotor activity. According to the latest Sanger SNP dataset, there are at least two non-synonymous coding polymorphisms in *Rufy1*, indicating a potential functional consequence. *Rufy1* encodes for an endosomal protein that is involved in endocytosis, making this an intriguing candidate. Furthermore, there is a single SNP in the 5′ UTR of *Hnrnph1* that could affect its expression. *Hnrnph1* encodes for a ribonuclear protein that binds RNAs and could influence pre-mRNA processing of a number of genes. We are currently conducting quantitative PCR experiments in congenic mice to determine if any of these three genes are differentially expressed - this would implicate the presence of *cis*-acting expression QTL(s) (eQTL) that regulate gene expression and behavior. Genomic analysis of gene expression will be used to identify potential gene networks modified by the 0.23 Mb region that may contribute to behavioral differences. We have clear evidence that at least one gene within the 0.23 Mb region contributes to the QTL. However, it is possible that additional, distal D2 allele(s) outside of this region are also required to fully account for the effect on phenotype. Definitive evidence for the sole involvement of this region necessitates further backcrossing to replace the distal D2 alleles with B6 alleles. Furthermore, an important consideration is that there are several intergenic and intronic SNPs within the 0.23 Mb region that could exert functional genomic effects. Last, structural differences or omissions in the annotation could underlie the importance of this region.
Keywords: QTL addiction genetic psychostimulant eQTL QTG
Disclosure: C. Bryant, Nothing to Disclose; C. Parker, Nothing to Disclose; M. Guido, Nothing to Disclose; L. Kole, Nothing to Disclose; J. Lim, Nothing to Disclose; G. Sokoloff, Nothing to Disclose; R. Cheng, Nothing to Disclose; A. Palmer, Nothing to Disclose.

M40. Kinase Inhibition within the Bed Nucleus of the Stria Terminalis Potentiates Binge Alcohol Intake by C57BL/6J Mice

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Background: A month-long history of binge alcohol drinking by C57BL/6J mice elevates indices of Group 1 metabotropic glutamate receptor (mGluR) signaling within both the nucleus accumbens and the central nucleus of the amygdala. Moreover, neuropharmacological inhibition of mGluR1/5 signaling through certain downstream kinases (e.g., PKC and PI3K) within both regions reduces binge alcohol intake. These data suggest that the maintenance of excessive alcohol intake might involve an increase in Group 1 mGluR signaling throughout the extended amygdala. To address this hypothesis, the present study employed a combination of immunoblotting and neuropharmacological approaches to examine the functional relevance of alcohol-induced changes in protein expression within the bed nucleus of the stria terminalis (BNST).
Methods: Male, C57BL/6J mice were trained to drink 20% alcohol or water for 2 hrs/day, under Drinking-in-the-Dark procedures. After 30 days of drinking, the BNST was dissected out and processed by conventional immunoblotting procedures for changes in Group 1 mGluRs, NMDA receptors, Homers, and several kinases found to be up-regulated by binge alcohol drinking within other extended amygdala structures. In a follow-up study, mice were surgically implanted with microinjector guide cannulae above the BNST and the effects of intra-BNST infusions of the PI3K inhibitors wortmannin (50 nM) and GDC-0941 (30 and 300 nM), as well as the ERK1/2 inhibitor U0126 (1-100 nM) upon binge alcohol drinking were assessed.
Results: Immunoblotting revealed relatively few changes in BNST protein expression in animals with a month-long history of binge alcohol drinking. Of the proteins examined, binge alcohol elevated Homer2a/b [$t(20) = 3.11$, $p = 0.005$], as well as the phosphorylated forms of ERK1/2 [$t(21) = 2.60$, $p = 0.02$] and PI3K [$t(19) = 2.18$, $p = 0.04$]. Inhibition of PI3K within the BNST using either a selective Class I inhibitor (GDC-0941) or a non-selective inhibitor (wortmannin) failed to alter alcohol drinking even when infused at doses well above their IC50's (repeated measures ANOVA, n.s.). In contrast, ERK1/2 inhibition with U0126 increased alcohol intake under our limited access procedures at doses 100-fold less than its IC50 [$F(3,18) = 5.68$, $p = 0.006$].
Conclusions: A chronic history of binge alcohol drinking elevates Homer2a/b and activates PI3K/MAP kinase pathways within the BNST, without influencing the expression of Group 1 mGluRs or NMDA receptors. While the functional relevance of binge drinking-induced increases in BNST Homer2 expression requires further study, the neuropharmacological data to date suggest an inhibitory role for ERK1/2 in the BNST in maintaining excessive alcohol intake.
Keywords: binge drinking, bed nucleus of the stria terminalis, alcoholism, ERK, Homer
Disclosure: M. Wroten, Nothing to Disclose; J. Courson, Nothing to Disclose; A. Williams, Nothing to Disclose; K. Szumlinski, Nothing to Disclose.

M41. Switching to Lurasidone in Patients with Schizophrenia: Tolerability and Effectiveness at 6 Weeks and 6 Months

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Background: In an effort to find the optimal regimen for an individual patient, switching between antipsychotic medications is common in the routine treatment of schizophrenia. The current short-term and extension studies evaluated the safety, tolerability and effectiveness of switching clinically stable, but symptomatic outpatients with schizophrenia or schizoaffective disorder to lurasidone.
Methods: Non-acute patients who met DSM-IV-TR criteria for schizophrenia or schizoaffective disorder and who were considered to be appropriate candidates for switching current antipsychotic medication due to insufficient efficacy and/or safety or tolerability concerns, were randomized to three open-label lurasidone switch strategies: a 40/40 group ($N = 74$) was started on 40 mg/d for 14 days; a 40/80 group ($N = 88$) was started on 40 mg/d for 7 days,

then increased to 80 mg/d for 7 days; and an 80/80 group (N = 82) was started on 80 mg/d for 14 days. The prior antipsychotic was tapered off over the initial 2-week study period (50% step-down end of week 1, discontinuation after week 2). All patients were then treated for 4 weeks with lurasidone flexibly dosed 40-120 mg/d. Patients were stratified based on whether the primary pre-switch antipsychotic was sedating (olanzapine, quetiapine) or non-sedating (all others). Time to treatment failure was evaluated as the primary objective of the study, prospectively defined as insufficient clinical response, exacerbation of underlying disease or discontinuation due to an adverse event (AE). Of the 198 subjects who completed the core 6 week study, 149 (75.3%) enrolled in an extension phase study and received 6 months of additional open-label, flexible-dose treatment with lurasidone (40-120 mg/day).

Results: Switching to lurasidone was well-tolerated with 198/229 (86.5%) of subjects completing the core 6-week study; 7.0% discontinued due to an AE. No clinically relevant differences in efficacy or tolerability were noted when comparing the 3 different switch strategies. Time to treatment failure was earlier in patients (35.8% of the total) who had been receiving a sedating antipsychotic prior to the switch to lurasidone compared with those who were receiving a non-sedating antipsychotic (log rank $p = 0.101$). Treatment with lurasidone in the core study was associated with LS mean (SE) within-group improvement at Week 6 on the PANSS total score (-5.3 ± 0.7; LOCF). Among subjects who entered the extension study, treatment with lurasidone was associated with additional LS mean improvement, from extension baseline at Month 6, of -3.6 ± 0.9 (OC; -1.5 ± 0.9, LOCF). Ninety-eight subjects (65.8%) completed the extension phase; premature discontinuation was most commonly due to withdrawal of consent (12.1%) or due to AEs (11.4%). Minimal median fasting changes (mg/dL), from core study baseline to Week 6 of the extension study, were observed on an LOCF analysis for total cholesterol (+1.0), triglycerides (−7.0) and glucose (−1.0). Mean change in weight was −0.3 kg at Week 6 (LOCF), with 1.8% of subjects experiencing a ≥7% reduction in weight, and 0.9% experiencing a ≥7% increase in weight. Minimal median fasting changes (mg/dL), from core study baseline to Month 6 of the extension study, were observed on an OC analysis for total cholesterol (−1.0), LDL cholesterol (−5.5), triglycerides (+2.0) and glucose (+2.0). Relative to core study baseline, mean change in weight was −0.7 kg at Month 6 (OC at Month 6), with 18.8% of subjects experiencing a ≥7% reduction in weight, and 16.0% experiencing a ≥7% increase in weight. The most frequent AEs during the core 6-week study were insomnia (8.8%), nausea (8.8%), akathisia (8.1%), and anxiety (6.1%). The most frequent AEs during the extension study were nausea (8.8%), dry mouth (4.7%), and vomiting (4.7%).

Conclusions: In this study and its extension, switching to lurasidone was well-tolerated regardless of initial dose or rate of titration. Patients switched to lurasidone generally demonstrated maintained or improved symptom control during core and extension treatment. At the end of 6 months of extension treatment, minimal changes were observed in median weight and lipid parameters, consistent with prior longer-term studies with lurasidone.

Keywords: schizophrenia, safety, lurasidone, switching, lipids

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M42. Neural Responses during Explicit and Implicit Face Processing Vary Developmentally in Bipolar Disorder

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Background: Despite compelling evidence that children and adults with bipolar disorder (BD) exhibit behavioral and neural

dysfunction during face emotion processing tasks (e.g., Brotman et al., 2008; 2010, Guyer et al., 2007; Kohler et al., 2011), only one prior study compared both age groups directly. Those results suggest that both pediatric and adult BD is characterized by amygdala dysfunction, but that in youth with BD, the dysfunction is evoked by a wider variety of emotional stimuli (Kim et al., 2012). The present study uses a parametric face emotion processing task to examine how age group and diagnosis influence the relationship between neural activity and increasing anger on a face. This design includes ecologically valid intermediate levels of emotion intensity and is relatively insensitve to amygdala habituation. A prior study using this parametric design and a partially overlapping sample of children found that healthy youths, but not those with BD, exhibit a positive linear trend between neural response in the amygdala and increasing anger intensity. Youths with BD exhibited a negative association between neural response in the posterior cingulate cortex (PCC) and increasing anger intensity (Thomas et al., in press). The present study extends this literature by examining developmental differences in neural dysfunction during a parametric face emotion processing task. We hypothesized that, in response to increasing anger on a face, both BD groups would exhibit a less positive linear trend in the amygdala, and a more negative linear trend in the PCC compared with healthy subjects. Based on a prior developmental study of BD (Kim et al., 2012), we also hypothesized that these abnormalities would be more pronounced in youths versus adults with BD.

Methods: 23 children with BD, 22 adults with BD, 27 healthy children, and 25 healthy adults viewed faces with increasing anger intensity [0% (neutral), 25%, 50%, 75%, and 100%] while making explicit (how hostile is the face?) or implicit (how wide is the nose?) emotional judgments about the face. Functional magnetic resonance imaging was used to examine linear trends between neural activation and increasing emotional intensity. Amygdala region of interest and whole brain analyses ($p < .005$; $k \geq 20$) were used to compare linear trends between participant groups and rating conditions.

Results: As predicted, both healthy children and adults exhibited positive linear trends between amygdala activation and increasing anger intensity ($ps < .05$). This effect was not present among either bipolar age group, suggesting that BD subjects fail to show appropriate modulation of amygdala activity in the presence of increasing anger. The whole brain analysis revealed a Diagnosis x Age Group x Rating Condition interaction in the bilateral anterior cingulate cortex (ACC) and bilateral PCC. Relative to healthy participants, BD youths exhibited more negative linear associations between activation in both regions and anger intensity in the nose width condition ($ps < .02$). While rating face hostility, the same pattern was seen in BD adults versus healthy adults in the ACC ($p < .02$) with a trend in the PCC ($p < .06$). Therefore, youths and adults with BD differed in the degree to which decreased activity in the ACC and PCC was associated with increasing anger intensity in the different rating conditions.

Conclusions: Relative to healthy participants, both children and adults with BD failed to modulate amygdala, ACC, and PCC activity in response to increasing anger intensity. However, in patients, the two rating conditions evoked important developmental differences in neural activity. The negative linear trend between activation in the ACC and PCC and increasing anger intensity was specific to the explicit condition for adults with BD and the implicit condition for children with BD. Together these findings suggest that BD patients are characterized by abnormal neural responses to emotional faces, but the nature of this dysfunction varies based on task demands and age. These findings also indicate how parametric designs, using different levels of emotion intensity, have the potential to highlight important neural patterns among BD youths and adults. Such distinctions may have treatment implications and warrant further study.

Keywords: bipolar disorder, emotion, face processing

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M43. Roles of Schizophrenia Susceptibility Gene KCNH2 3.1 Isoform in Regulation of Neuronal Excitability, Long-term Potentiation, Synaptogenesis and Cognition

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Background: Abnormal neuronal activities mediated by dopamine, glutamate or GABA are key common component of leading hypotheses of schizophrenia. Our recent discovery of a novel schizophrenia susceptibility gene, KCNH2, provides some direct evidence of abnormal neuronal activity as a fundamental problem in schizophrenia that would influence DA, GLU and GABA neurons. KCNH2 is a voltage-gated potassium channel critical for repolarization of neurons and cardiac myocytes. A novel primate-specific and brain-selective isoform of KCNH2, KCNH2 3.1, has been identified. It is expressed at higher levels in the postmortem brain of schizophrenic patients than those of normal controls and is linked to risk associated genotypes. The most remarkable feature of KCNH2 3.1 is that it lacks the first two exons encoding the PAS domain of the full length KCNH2, an important domain for slow deactivation during repolarization. Without the PAS domain, KCNH2 3.1 closes the channel too quickly during the deactivation phase, and increases the excitability and firing frequency of neurons. This effect was confirmed in primary neuronal culture transfected with KNCH2 3.1.

Methods: To test the neuronal excitability hypothesis on schizophrenia, we developed inducible transgenic mice carrying the human KCNH2 3.1 transgene. The transgenic mice were analyzed by quantitative RT-PCR, electrophysiological whole-cell recording, patch clamp recording, confocal microscopy of synaptogenesis, and T-maze working memory test.

Results: Quantitative RT-PCR analyses of gene expression showed high levels of KCNH2 3.1 mRNA in frontal cortex and hippocampus of the transgenic mice. Electrophysiological analyses of cortical neurons in brain tissue sections revealed shortened tail currents and increased excitability of layer V neurons in frontal cortex. Analyses of hippocampal neurons in the CA1 region of the transgenic mice showed that the long-term potentiation (LTP) was impaired in young but not adult mice. Confocal microscopic analyses of synaptogenesis in cortical neurons demonstrated that the number of mature mushroom-shaped synapses was significantly decreased in the transgenic mice, which was partially reversed by *in vivo* treatment with clozapine, an effective antipsychotic drug and also a potent KCNH2 antagonist. Behavioral analyses of the transgenic mice revealed deficits in working memory and in spatial memory, consistent with the physiological changes.

Conclusions: Our results suggest that increased neuronal excitability related to KCNH2 3.1 activity leads to decreased mature synapses on pyramidal neurons and impaired cognitive function referable to prefrontal and hippocampal circuitry. These findings may characterize pathophysiological changes underlying the cognitive dysfunction in schizophrenia. Specific modulators of KCNH2 3,1 might improve cognitive function in patients and represent a new class of antipsychotic drugs.

Keywords: schizophrenia, KCNH2, cognition, synaptogenesis, excitability

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M44. Neuronal Systems Underlying the Antidepressant Response to Ketamine

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Background: The NMDA receptor antagonist, ketamine, has been demonstrated to produce a rapid and sustained antidepressant response in preclinical rodent models as well as in depressed patients; however, the mechanisms underlying this effect have not been conclusively demonstrated. Preclinical studies have suggested that low-dose ketamine administration induces a molecular cascade, including activity-dependent BDNF release, leading to neuroplastic changes thought to underlie antidepressant efficacy. Here we examine the role of the ventral hippocampus (vHipp) and medial prefrontal cortex (mPFC) in the antidepressant response to a sub-anesthetic dose of ketamine.
Methods: Male Sprague Dawley rats were implanted with stainless steel cannulae targeting the vHipp and administered either lidocaine (2%, 0.5 ml) or vehicle directly into the vHipp followed immediately by the systemic administration of a low-dose of ketamine (10 mg/kg i.p.) or vehicle. The forced swim test was utilized as a putative measure of antidepressant efficacy 30 mins, 24 hours and 1 week following drug treatment. Given the reported role of BDNF in the antidepressant response to ketamine, we examined the phosphorylation state of the BDNF receptor, TrkB, postmortem using phospho-specific antibodies to tyrosine 515, 705 and 816. We have previously demonstrated that vagal nerve stimulation (VNS), an effective therapy in treatment-resistant patients, differentially phosphorylates distinct sites of the TrkB receptor when compared to conventional antidepressant medications. In a separate experiment, still in progress, the effects of ketamine on stress-induced deficits in cognitive flexibility were examined as a behavioral readout of prefrontal cortical function that is sensitive to antidepressant treatment. Rats exposed to chronic unpredictable stress or unstressed controls were given an injection of vehicle or ketamine (10 mg/kg i.p.) 24 hr prior to testing for cognitive flexibility on an attentional set-shifting test (AST).
Results: Transient, lidocaine-induced inactivation of the vHipp inhibited the persistent (i.e., one week) but not the acute (i.e., 30 min) antidepressant-like effects of ketamine as measured by immobility on the forced swim test. Further, acute ketamine administration induced hippocampal phosphorylation of tyrosines 705 and 816 of the TrkB receptor, and subsequent activation of downstream signaling molecules that may underlie the plastic changes associated with antidepressant efficacy. Acute VNS, however, also caused phosphorylation of an additional site, Y515, on TrkB.
Conclusions: Taken together we provide a multidimensional approach aimed at better understanding the brain regions and molecular mechanisms underlying the antidepressant response to ketamine. Specifically, we demonstrate that low-dose ketamine results in hippocampal TrkB receptor phosphorylation and, moreover, that hippocampal activity may contribute to the sustained antidepressant actions of ketamine.
Keywords: ketamine, antidepressant, hippocampus, prefrontal cortex, BDNF

Disclosure: D. Lodge, **Part 1:** Consutant - Dey Pharmaceuticals; F. Carreno, Nothing to Disclose; A. Shah, Nothing to Disclose; J. Jett, Nothing to Disclose; P. Delgado, Nothing to Disclose; D. Morilak, **Part 1:** Consultant for Dey Pharmaceuticals (now Mylan), **Part 4:** Research funding from Forest Labs and Lundbeck; A. Frazer, **Part 1:** Consultant for Dey Pharmaceuticals (now Mylan). On advisory boards for Lundbeck/Takeda and for Lilly.

M45. A Novel Serotonin-2 (5-HT2) Modulator as a Candidate Drug to Treat Impulsive Behavioral Disorders and Psychoses without Weight Gain as a Side Effect

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Background: It is hypothesized that the desired serotonin 5-HT2 receptor pharmacology required to treat impulsive behavioral disorders, including, psychostimulant abuse/addiction and binge eating, as well as, psychoses, is 5-HT2A antagonism and/or 5-HT2C agonism. Currently, however, no selective 5-HT2A antagonist has demonstrated clinical efficacy to treat psychostimulant addiction or psychoses, and a clinically-acceptable 5-HT2C agonist that does not also activate 5-HT2A (hallucinations) and/or5-HT2B (cardiopulomonary toxicity) receptors has not been reported. Negative modulation of amphetamine-induced locomotor activity in rodents is a commonly used first-stage screen for new drugs to treat amphetamines abuse/addiction, and, all effective antipsychotic compounds attenuate the robust head-twitch reponse (HTR) in mice elicited by the 5-HT2 agonist (−)−2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI), as well as, the hyperactivity elicited by the NMDA antagonist MK-801 and/or amphetamine.
Methods: The (−) and (+) enantiomers of a novel compound, (2S, 4R)-*trans*-4-(3′-bromophenyl)-*N,N*-dimethyl-2-aminotetralin (*meta*-bromo-PAT or *m*- Br-PAT), were synthesized, characterized *in vitro* for binding and function at mouse and human 5-HT2A, 2B, and 2C receptors. Subsequent studies in male C57Bl/6J mice examined these compounds in animal models predictive of activity to modulate amphetamine-induced behaviors, antipsychotic activity, and in a model of compulsive (binge) eating.
Results: Both (−) and (+)-*m*-Br-PAT bound to mouse and human 5-HT2 receptors with the (−) enantiomer being approximately 16-, 5- and 27-fold more potent at 2A, 2B, and 2C receptors respectively. (−)-*m*-Br-PAT was a relatively high efficacy 5-HT2C agonist measured by functional activation of PLC assessed by [^{3}H]-IP formation in HEK cells transiently expressing 5-HT2C receptors. Both enantiomers attenuated the HTR elicited by DOI (1.0 mg/kg) with (−)-*m*-Br-PAT being approximately 3-fold more potent. (−)-*m*-Br-PAT completely blocked MK-801-elicited (0.3 mg/kg) increases in locomotor activity, and was behaviorally active for at least 2 hours. Hyperactivity produced by amphetamine (3.0 m/kg) administration was similarly attenuated by (−)-*m*-Br-PAT. Consumption of a highly palatable food in non-food-deprived mice was decreased by ∼65% and 45% following administration of the (−) and (+) enantiomers, respectively.
Conclusions: Here we report the pharmacological and initial behavioral characterization of two enantiomers of a novel 5-HT2A/2B antagonist with 5-HT2C agonist activity that may have therapeutic efficacy to treat impulsive and compulsive behavioral disorders such as psychostimulant (amphetamines) abuse/addiction, as well as, binge eating, and, to treat psychoses without the adverse side effect of weight gain.
Keywords: animal models antipsychotic medication development serotonin 2C agonist serotonin 2A antagonist
Disclosure: D. Morgan, Nothing to Disclose; C. Canal, Nothing to Disclose; K. Kondabolu, Nothing to Disclose; R. Sakhuja, Nothing

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M46. Relationship of Plasma Oxytocin Levels to Baseline Symptoms and Symptom Changes During Oxytocin Administration in Men and Women with Schizophrenia

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Background: Ongoing and completed randomized clinical trials administering intranasal oxytocin in individuals with schizophrenia suggest short-term benefits for both positive and negative symptoms. Improvements in these symptoms are demonstrated in both individuals with high levels of baseline paranoia (Feifel et al. 2010; Pederson et al. 2011) and those with general symptoms (Kelly, Lee et al., unpublished). Although individuals with schizophrenia and healthy controls may not differ in either central or peripheral levels of oxytocin, these levels have been associated with clinical symptoms in schizophrenia patients. For example, lower central oxytocin levels are associated with a greater degree of negative symptoms in men ($n = 27$) with schizophrenia (Sasayama et al 2012). Rubin et al (2011) found peripheral levels of oxytocin are associated with less severe positive symptoms and general psychopathology in women ($n = 23$), but not in men ($n = 27$), with schizophrenia (Rubin et al. 2011). No studies have been published examining changes in peripheral oxytocin levels and symptoms during repeated treatment with intranasal oxytocin in schizophrenia.
Methods: We report baseline oxytocin values in both men and women (total $N = 27$) as well as the correlation between oxytocin levels and positive and negative symptoms (measured by the Brief Psychiatric Rating Scale (BPRS) and Scale for the Assessment of Negative Symptoms (SANS) during a 3 week double blind placebo controlled trial of intranasal oxytocin (20 IU BID). Plasma oxytocin levels were drawn at baseline and endpoint and analyzed by specific RIA following acetone-ether extraction (Verbalis et al 1986). The minimum detectable concentration of oxytocin in plasma was 0.25 pg/ml. The oxytocin antiserum (Pitt-Ab) displayed $<1\%$ cross reactivity with vasopressin.
Results: Mean oxytocin levels in the entire sample ($N = 27$) at baseline were 1.1 ± 0.4 pg/ml and did not differ between males (1.1 ± 0.4; $N = 19$) and females (1.1 ± 0.5; $N = 8$). At baseline, no significant correlations between peripheral oxytocin levels and positive or negative symptoms were found, either in the total sample or by gender. From baseline to endpoint there was no significant increase in oxytocin levels in the group administered oxytocin (1.29 ± 0.48 to 1.46 ± 0.69 pg/ml, change $= 0.17$ pg/ml) compared to those administered placebo (0.96 ± 0.36 to 1.01 ± 0.41 pg/ml; change $= 0.05$ pg/ml). However, during the 3 week trial, we found significant correlations between increased oxytocin levels and improvement in BPRS total score ($r = -0.573$, $p = 0.04$) and BPRS negative symptom factor ($r = -0.572$, $p = 0.04$) in those receiving oxytocin demonstrating that improvements in these symptoms were associated with increases in peripheral oxytocin levels. There was also a trend, in the oxytocin group only, for the improvement in SANS total score to correlate with an increase in oxytocin levels ($r = -0.46$, $p = 0.084$). No effect was found in the placebo group. The significant correlations between changes in oxytocin levels and changes in symptoms did not differ as a function of sex. However, the results in the oxytocin group were driven by two participants with relatively large changes in oxytocin levels and either the BPRS total score or BPRS negative symptom factor.
Conclusions: Contrary to previous studies, we did not find associations between peripheral oxytocin levels and symptomatology in our baseline data. Interestingly, following a 3 weeks of intranasal oxytocin, we found that increases in peripheral oxytocin levels were associated with greater improvements in negative symptoms and total symptomatology. More work is needed to understand the relationship of peripheral oxytocin levels as measures of symptom or symptom response in both men and women.
Keywords: oxytocin, schizophrenia, negative symptoms
Disclosure: D. Kelly, **Part 4:** Bristol Myers Squibb and Ameritox Ltd.; H. Wehring, Nothing to Disclose; R. McMahon, Nothing to Disclose; F. Liu, Nothing to Disclose; J. Linthicum, Nothing to Disclose; J. Verbalis, Nothing to Disclose; R. Buchanan, **Part 1:** Advisory Board: Amgen; Astellas; Janssen Pharmaceuticals, Inc.; NuPathe, Inc.; Pfizer; Roche; Takeda, Consultant: Abbott; Amgen; Bristol-Meyer-Squibb; EnVivo; Pfizer; Takeda; , DSMB member for Pfizer and Otsuka; G. Strauss, Nothing to Disclose; L. Rubin, Nothing to Disclose; M. Lee, Nothing to Disclose.

M47. Drug-unpaired Environments Regulate Dendritic Spine Dynamics in the Nucleus Accumbens

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Background: Repeated exposure to amphetamine leads to the development of biochemical and neuroanatomical phenotypes that may underlie the formation of associative drug conditioning. Brief elevations in c-Fos expression in nucleus accumbens (NAcc) neurons following re-exposure to a drug-paired, but not unpaired, environment suggest that expression of this immediate early gene serves as a marker for neurons which process drug-context information. This correlation is supported by evidence showing that prevention of conditioning inhibits environmental regulation of c-Fos expression. In contrast, ΔFosB displays a long-lasting context-independent increase following repeated drug exposure, a neuronal expression pattern that overlaps with c-Fos activation following exposure to drug-paired stimuli. When conditioning accrues, a proportion of ΔFosB(+) cells may process this information although ΔFosB levels are also increased if conditioning is prevented. This transcription factor may thus have various functions and serve as a marker for cells encoding other information such as non-associative behavioral sensitization that can manifest in the absence of associative conditioning. Previous research has revealed brain-region specific changes in dendritic architecture following repeated systemic amphetamine administration. Given the well documented relationship between conditioning and dendritic spine growth, we hypothesized that dendritic changes may be restricted to c-Fos(+) and ΔFosB(+) neurons that encode this behavioral information.
Methods: Labeled cells were injected with the carbocyanine neuronal tracer DiI in order to characterize their dendritic spine morphology.
Results: One week after withdrawal from repeated amphetamine, NAcc FosB(+) cells showed an increase in the basal frequency (no challenge) of spines with medium head diameters. This increase was absent in c-Fos(+) neurons 30-minutes after re-exposing rats to a drug-unpaired environment, but preserved after exposure to a drug-paired environment. Consistent with these results, significant decreases in levels of the actin binding proteins Arp2 and p-cofilin were observed following exposure to the drug-unpaired environment.
Conclusions: These results support the existence of long-lasting conditioning-related changes in the dendritic spine properties of NAcc neurons following repeated amphetamine and the inhibitory regulation of these changes by drug-unpaired environments.
Keywords: amphetamine conditioning, c-Fos, dendritic spines, FosB, nucleus accumbens

Disclosure: P. Vezina, Nothing to Disclose; B. Singer, Nothing to Disclose; N. Bubula, Nothing to Disclose; V. Bindokas, Nothing to Disclose.

M48. Understanding the Role of G-protein Dependent Signaling in the Indirect Striatal Pathway in Behavioral Inhibition Using Targeted Viral-mediated Gene Transfer of DREADD Receptors

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Background: Behavioral inhibition, which allows individuals to interrupt a pre-planned action, is compromised in a number of neuropsychiatric disorders, including obsessive-compulsive disorder, attention deficit/hyperactivity disorder (ADHD), and drug addiction. Dopamine D_2 receptor signaling in the striatum has been found to regulate behavioral inhibition; however, striatal D_2 receptors are located on both the GABAergic medium spiny projection neurons of the indirect pathway and the cholinergic interneurons. Which population of dopamine D_2 receptor-containing neurons regulates this process has not yet been examined.
Methods: To begin to address this question, we used a novel chemical-genetic approach involving cell-specific viral-mediated gene transfer of engineered G-protein coupled DREADD (Designer Receptor Exclusively Activated by a Designer Drug) receptors. Because DREADD receptors are only activated by the otherwise pharmacologically inert synthetic ligand clozapine-*N*-oxide (CNO) and the enkephalin promoter was used to target transgene expression, this strategy allows us to mimic D_2 receptor function exclusively in indirect pathway neurons. Using this procedure, we examined how altering G-protein receptor signaling in indirect pathway neurons of the dorsomedial striatum of Long Evans rats altered task performance in one common model of behavioral inhibition, the stop-signal task.
Results: We found that activating $G_{i/o}$-coupled DREADD receptors in indirect pathway neurons of the dorsomedial striatum during the presentation of stop-signal delays increased stop-trial accuracy without effecting go-trial accuracy or mean reaction speed. Increasing $G_{i/o}$-mediated signaling also increased stop-signal reaction time (SSRT), which reflects the speed of the inhibition process. Experiments are currently underway to determine if activating G_s-coupled receptors in indirect pathway striatal neurons produces the opposite effect.
Conclusions: These findings demonstrate that dopamine D_2 receptor-like activity in indirect pathway neurons is sufficient to enhance performance in a stop-signal task and suggest that altered G-protein coupled signaling in the indirect pathway is likely to be a key mechanism underlying the deficits in behavioral inhibition that are observed in impulsive individuals.
Keywords: stop-signal task, rat, striatum, indirect pathway, DREADD
Disclosure: S. Ferguson, Nothing to Disclose; D. Newcomer, Nothing to Disclose; T. Robbins, **Part 1:** Cambridge Cognition, Lilly, GlaxoSmithKline, Merck, Shire Pharmaceuticals, Lundbeck, **Part 4:** Lilly, Lundbeck, GSK; J. Neumaier, Nothing to Disclose.

M49. Antipsychotic-like Actions of Amylin in Ventral Striatal Regions Enriched in RAMP-1 Gene Expression

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Background: There is considerable translational potential in identifying understudied neuromodulator systems that mediate antipsychotic-like effects, but which may lack the side effect profile associated with current antipsychotic drugs. The present study represents an exploration of the AMY_1 receptor, a high-affinity receptor for CGRP-related peptides such as amylin (AMY) and salmon calcitonin. This receptor is highly concentrated in the ventral striatum, with particularly high densities found in the nucleus accumbens shell (AcbSh). Because the Acb shell is a crucial neural substrate for antipsychotic actions, and because AMY infusion into the Acb shell produces some behavioral effects reminiscent of functional dopamine antagonism, we explored possible antipsychotic-like effects of AMY_1 receptor manipulations in the Acb shell using the prepulse inhibition (PPI) paradigm. PPI refers to the normal diminution of the startle response when a barely detectable prepulse is presented immediately prior to an intense startling stimulus ('pulse'), and is perhaps the most extensively validated operational measure of the sensorimotor gating abnormalities that are seen in schizophrenia and thought to contribute to the impaired information-filtering of this illness. In conjuncton with PPI studies, we have also begun a detailed anatomical mapping of the expression patterns of genes related to AMY_1 receptor function. These include Receptor Activity Modifying Proteins (RAMPs), which regulate the ligand binding affinity of the "template" CGRP receptor genes CL and CR; RAMP-1 association with CT produces the high-affinity AMY_1 receptor. We mapped the regional expression patterns of all these genes, and asked whether they are developmentally regulated during adolescence (a period of great vunerability for the emergence of psychosis), in a manner similar to dopamine receptor genes in the ventral striatum. An important impetus for this work is the observation that AMY and related peptides produce satiety-like effects; hence, drugs that target this system may have antipsychotic efficacy without weight-gain liability and metabolic syndrome, which are major side effects of current antipsychotic medications.
Methods: Separate groups of male Sprague-Dawley rats (N = 8-10 per group) equipped with bilateral stainless steel cannulae aimed at either the nucleus accumbens shell (AcbSh) or caudate received microinfusions of amylin (0, 30, or 100 ng/0.5 ul) along with systemic injection of amphetamine (AMPH: 0 or 1.75 mg/kg) prior to testing in startle chambers for PPI. In a separate experiment, the effects on PPI of intra-AcbSh infusion of a selective antagonist for AMY_1 receptors, AC187 (0, 10, or 20 ug/0.5 ul), was studied. Startle chambers consisted of clear Plexiglas cylinders housed inside of a sound-attenuated, lighted, ventilated cabinet with ceiling-mounted speakers; computer interface produced all the stimuli and recorded resultant startle responses. The test session consisted of (presented in a randomized order after a 5-min acclimation period with only the 65-decibel background noise) trials in which startling stimuli (120-dB) were presented either alone ('pulse-alone') or 100 msec after non-startling prepulses that were 3, 9, or 15 dB above the background noise; trials with no stimuli were also included. The startle reponse to each trial was recorded, and PPI was calculated as % PPI = 100 – {[(startle response for Prepulse + Pulse trial)/(startle response for Pulse-Alone trial)] x 100} for each prepulse intensity. Baseline startle represented average startle magnitude of all the Pulse-Alone trials. Finally, gene expression profiles in AcbSh using *in situ* hybridization were obtained for RAMP-1, RAMP-2, RAMP-3. These genes along with the CT and CL receptor genes were mapped throughout striatal compartments, with particular emphasis on the core and shell subdivisions of the nucleus accumbens.
Results: AMPH caused a schizophrenia-like disruption of PPI. While amylin itself had no effects on PPI, when infused into AcbSh, amylin significantly reversed AMPH-induced PPI deficits. In contrast, amylin infusion into neighboring areas of caudate had no effect on basal or AMPH-disrupted PPI. Therefore, stimulation of AMY_1 receptors in AcbSh can normalize PPI deficits induced by psychotomimetic drugs, perhaps through modulating transmission of dopamine or norepinephrine, which are released in this site and

known to disrupt PPI via AcbSh. Conversely, blockade of AMY_1 receptors in Acb shell with the selective antagonist AC187 caused a significant reduction in PPI, suggesting that basal amylin tone at these receptors modulates this important schizophrenia-related behavior. All effects on PPI were independent of changes in baseline startle reactivity, and thus represent true effects on sensorimotor gating. Initial findings from the mapping of AMY-related genes indicate a selective striatal expression of RAMP-1 (the RAMP that confers high ligand affinity to CT, producing the AMY_1 receptor) relative to the other RAMPs, with a particularly high densitiy in the medial Acb shell. This is the same area in which local AMY infusions reversed the PPI-disruptive effects of amphetamine, and where blockade of these receptors disrupted PPI. Finally, we preliminarily have determined that RAMP-1 expression is higher in adolescent rats relative to adults; we are following this up with developmental analyses of the other RAMP genes, as well as genes encoding CL and CR.

Conclusions: The AMY_1 receptor shows promise as a target for the development of new antipsychotic drugs, or for pharmacological adjuncts that augment antipsychotic actions while delimiting or preventing weight-gain side effects. Moreover, our preliminary gene expression analysis suggests that levels of RAMP-1 gene transcription changes through adolescence. This observation, together with the present finding that blocking endogenous ligand "tone" at AMY1 receptors disrupts PPI, suggests that AMY1 signalng may represent an important modulatory influence in the striatum throughout adolescence– possibly "buffering" dopamine tone, which is known to be upregulated during adolescence.

Keywords: prepulse inhibition startle dopamine schizophrenia nucleus accumbens

Disclosure: V. Bakshi, Nothing to Disclose; S. Baisley, Nothing to Disclose; Q. Bremer, Nothing to Disclose; B. Baldo, Nothing to Disclose.

M50. Role of Glycogen Synthase Kinase-3Beta and Beta-Catenin in the Ventral Tegmental Area in Stress and Anxiety Behaviors

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Background: Major depressive disorder is a prevalent psychiatric syndrome, yet despite its widespread occurrence the underlying molecular mechanisms remain poorly understood. To examine the neuronal circuits and molecular pathways that contribute to depression, we use the chronic social defeat stress (CSDS) mouse model, which recapitulates multiple depressive-like behaviors that display a sensitivity to chronic, but not acute, antidepressant treatment. CSDS mice exhibit social avoidance and decreased sucrose preference, among other abnormalities. We have found that alterations in the mesolimbic pathway, specifically dopamine (DA) neurons in the ventral tegmental area (VTA) that project to the nucleus accumbens (NAc), contribute to both the behavioral changes induced by CSDS and antidepressant action. Specifically, we have shown that decreased signaling of AKT, a specific molecular pathway downstream of brain-derived neurotrophic factor (BDNF), within the VTA plays a critical role in CSDS-induced behavioral changes. One target of AKT phosphorylation is the glycogen synthase kinase 3b (GSK3b). Given previous data from our group showing that altered GSK3b activity in the NAc can influence depressive-like behaviors, we sought to determine whether modulation of GSK3b activity in the VTA played a role in CSDS-induced behaviors. In addition to the decreased AKT signaling in the VTA, the firing rate of VTA DA neurons is increased in animals that are susceptible to CSDS. We have shown previously that chronic opiate administration decreases the soma size of DA neurons within the VTA and that this morphological change, is mediated by decreased neurotrophic factor signaling and increased neuronal activity. Given the similarity of changes induced in the VTA by chronic opiates and social stress, we wanted to determine whether DA cell size was altered in response to CSDS and whether morphological changes were dependent on altered AKT/GSK3b activity.

Methods: We employed the previously validated chronic social defeat paradigm. Briefly, C57bl6 mice were subjected to a 10 min. social defeat episode with a novel CD1 mouse for 10 days. Following each defeat episode the C57 mouse was separated from the CD1 mouse. After the tenth defeat, the mice were singly housed and social avoidance will be tested 24 hours later. Mice underwent a battery of behavioral measures including social avoidance, sucrose preference, elevated plus maze, open field, and the forced swim test. For all surgical procedures, bilateral herpes simplex virus (HSV) infusions (0.5 ml) targeting the VTA were used and mice were analyzed 2-5 days following surgery, the peak expression timeline for HSV studies. Immunohistochemistry and morphological analysis was completed using published protocols. Briefly, mice were transcardially perfused with 4% paraformaldehyde, brains were post-fixed and cryoprotected and 30 μM sections containing VTA were collected for analysis. Tysrosine hydroxylase immunoreactivity was used to identify DA neurons within in the VTA and confocal microscopy was used for imaging followed by Volocity software for 3D reconstruction to determine DA soma size.

Results: Using herpes simplex virus to locally overexpress a dominant-negative GSK3b mutant in the VTA, we are able to reverse CSDS-induced social avoidance. Additionally, HSV-mediated overexpression of wild-type GSK3b in the VTA appears to increase social avoidance to a sub-threshold social defeat and increase immobility time in the forced swim test. These data are consistent with CSDS-induced decreases in VTA AKT activity, which would be expected to increase GSK3b activity via decreased inhibitory phosphorylation. Increased GSK3b activity within the VTA would be predicted to decrease levels of the transcription factor beta-catenin (bCat) and, indeed, we find decreased bCat mRNA in the VTA following CSDS. While the role played by VTA bCat activity in CSDS is not yet clear, we have found that HSV-mediated overexpression of wild-type bCat in this region does alter behavior. Specifically, increased VTA bCat activity appears to be anxiolytic, as HSV-bCat mice spent more time in the open arm of the elevated plus maze and in the center of the open field arena.

We also have preliminary data that CSDS induces a similar ($\sim$25%) decrease in the soma size of VTA DA neurons 48 hours after the last stress. We plan to examine the time-course of this morphological change to determine whether it correlates with the development of behavioral abnormalities. We are also in the process of determining with the morphological change is dependent on AKT and/or GSK3b activity.

Conclusions: Together, these data suggest that altered activity of the AKT-GSK3b-bCat signaling pathway within the VTA mediates changes in stress- and anxiety-related behaviors and may serve as a promising target for novel therapeutic agents. Further, these signaling changes may induce structural changes in VTA DA neurons that contribute to the maintenance of the phenotype. Overall, these studies seek to identify and characterize neuroadaptations in the mesocorticolimbic circuit induced by stress with the ultimate goal that a more thorough understanding of the molecular underpinnings of this stress-induced morphological change may illuminate novel antidepressant targets.

Keywords: dopamine, ventral tegmental area, GSK3, morphology, Beta catenin

Disclosure: M. Mazei-Robison, Nothing to Disclose; R. Appasani, Nothing to Disclose; C. Dias, Nothing to Disclose; R. Neve, Nothing to Disclose; E. Nestler, Nothing to Disclose.

M51. Deficient Prepulse Inhibition in Schizophrenia Detected by the Multi-site Consortium on the Genetics of Schizophrenia (COGS)

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Background: Startle and its inhibition by weak lead stimuli (prepulse inhibition: PPI) are studied to understand the biology of information processing in patients and healthy comparison subjects (HCS). PPI has a strong genetic basis in infrahumans, and there is evidence for its heritability, stability and reliability in humans. The NIMH COGS is a multi-site effort to leverage these properties of PPI and other putative "endophenotypes" to identify risk genes for schizophrenia (SZ). Using a family-based ascertainment strategy ("COGS-I"), significant associations were identified between PPI and single nucleotide polymorphisms (SNPs) in SZ probands and unaffected family members, and linkage analyses provided additional evidence for the genetic basis for PPI deficits in SZ. These findings are now being extended in a large study of patients and unrelated HCS ("COGS-II"; n = 3000), using 5 geographically dispersed test sites to accommodate the need for a large study sample. Here, we describe our first "interim analysis" of 750 study subjects.
Methods: 750 carefully screened SZ patients and HCS completed PPI testing as part of a test battery including other neurophysiolgical and neurocognitive measures, with blood acquisition for genetic analysis. Inclusion/exclusion criteria, assessment instruments and PPI methods are as per published reports; testers complete rigorous annual training and all methods undergo careful quality assurance. Bilateral eyeblink startle responses were acquired using EMG of orbicularis oculi. As in published COGS reports, PPI was elicited using 115 dB(A) 40 ms noise startle bursts and 20 ms 15 dB prepulses over a 70 dB(A) noise background. Prepulses of 30, 60 and 120 ms onset asynchrony were used; in all COGS analyses, PPI with 60 ms prepulse intervals is the primary dependent measure for PPI. Planned analyses of PPI assess effects of diagnosis, sex and test site; secondary analyses focus on known PPI-modifying variables, including medications and smoking; tertiary analyses assess relationships between PPI and other neurophysiological and neurocognitive measures. Genomic analyses will be conducted with the full study sample.
Results: Of the 750 subjects (ss) completing startle testing, 629 met established response criteria for PPI analysis (mean startle magnitude in the first PPI trial block $\geq$ 10 units). Of these 629 ss, 267 were HCS (M:F = 127:140; age (y) mean (SD) = 37.40 (13.91)), and 362 were SZ patients (M:F = 260:102; age (y) mean (SD) = 45.31 (11.14)); compared to HCS, patients were significantly older, and more likely to be male. Among patients, 7.5% took no antipsychotics (APs), 8.5% took only 1st-generation APs, and 84% took 2nd generation APs. Among HCS, analysis of 60 ms PPI revealed no significant effect of test site, though the range across the 5 sites (mean (SD)) was 48.66 (34.04) (site 5) – 58.29 (27.95) (site 2)). ANOVA including SZ patients revealed a significant effect of diagnosis (HCS > patients; $p < 0.003$), no significant effect of test site or diagnosis x site interaction ($F < 1$), and a near-significant effect of sex (M > F; $p = 0.06$), with no other informative effects. PPI was arithmetically reduced among patients vs. HCS at all 5 sites. Despite this highly significant effect of diagnosis and lack of diagnosis x site interaction, effect sizes (d) for PPI deficits in patients at sites 1–4 were 0.35, 0.33, 0.22, 0.20, while for site 5, it was 0.01. A more inclusive analysis of PPI at 30, 60 and 120 ms intervals confirmed our previously reported main effect of sex (M > F, $p < 0.03$) and interaction of diagnosis and interval ($p < 0.0001$), with no interaction of site ($F < 1$) and maximum SZ-related PPI deficits at the 60 ms interval. Analysis of startle magnitude on pulse-alone trials revealed significant effects of site ($p < 0.006$) and diagnosis (HCS > patients; $p < 0.02$). Inspection revealed no significant effects of diagnosis on startle magnitude at test sites 1–4 with robust HCS > patient PPI differences, but a significant effect of diagnosis on startle magnitude (HCS > patients; $p < 0.01$) at the single test site (5) lacking significant HCS > patient PPI differences, and also having the lowest HCS PPI values. Secondary and tertiary analyses will be presented.
Conclusions: This interim analysis confirmed the ability to detect and study significant PPI deficits in SZ patients vs. HCS among COGS-II subjects tested in a multi-site platform. PPI patterns previously reported in single-site studies related to diagnosis, prepulse intervals and sex effects were also reproduced in this interim multi-site database. The mean effect size among sites 1–4 (d = 0.27) is consistent with the predicted impact of atypical APs on this measure, and exceeded our published, single-site value for 60 ms PPI in a sample with comparable atypical AP use (d = 0.24; Arch Gen Psychiat 63:1325-1335, 2006); potential contributors to the differences in PPI and startle magnitude detected at site 5 – including subject demographics, clinical characteristics and testing methodologies – are being examined. More generally, the target SZ "endophenotype" of reduced PPI detected in these studies should provide a valuable tool for identifying and confirming SZ risk genes in future COGS-II analyses. Support: MH065571.
Keywords: schizophrenia, prepulse inhibition, endophenotype, genetics, multi-site
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M52. Pain-related Depression of the Mesolimbic Dopamine System in Rats: Expression, Blockade by Analgesics, and Role of Endogenous Kappa Opioids

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Background: Pain is often associated with depression of behavior and mood, and relief from pain-related depression is a common goal of treatment with analgesic drugs. This preclinical study tested the hypothesis that pain-related depression of behavior in rats results from activation of endogenous kappa opioidergic systems and subsequent kappa receptor-mediated inhibition of mesolimbic dopamine neurons. We have reported previously that a common visceral noxious stimulus, intraperitoneal (IP) injection of dilute acid, produces an analgesic-reversible depression of operant responding in an assay of intracranial self-stimulation (ICSS) in rats. The present study compared effects of IP acid and the exogenous kappa agonist U69593 on ICSS and on microdialysis measures of mesolimbic nucleus accumbens dopamine levels in rats. The nonsteroidal anti-inflammatory drug ketoprofen, the mu opioid receptor agonist morphine, and the kappa opioid receptor antagonist norbinaltorphimine were then evaluated for their ability to block acid- and U69593-induced depression of ICSS and nucleus accumbens dopamine. Our hypothesis predicted that both IP acid and U69593 would depress ICSS and nucleus accumbens dopamine levels, and that effects of acid would be blocked by analgesics and by the kappa antagonist.
Methods: Adult male Sprague-Dawley rats were prepared either with intracranial electrodes targeting the medial forebrain bundle (for behavior studies of intracranial self-stimulation) or cannulae targeting the nucleus accumbens (for microdialysis studies of mesolimbic dopamine). Rats in behavioral studies were trained to lever press under a fixed-ratio 1 schedule for electrical brain stimulation, and daily experimental sessions were composed of

multiple 10 min components. During each component, the frequency of brain stimulation was systemically varied from 158-56 Hz in ten 0.05 log unit steps, and the primary dependent variable was the total number of stimulations delivered across all frequencies. On test days, ICSS components were conducted before and after experimental treatments, and ICSS data determined after each treatment were expressed as a percent of the baseline data collected before treatment on that day. Rats in neurochemical studies were fitted with microdialysis probes on the day of the experiment, and samples were collected at 6-min intervals before and after experimental treatments. The primary dependent variable was the concentration of dopamine in each sample determined by high performance liquid chromatography coupled to electrochemical detection. Dopamine levels determined after each treatment were expressed as a percent of the baseline data collected before treatment on that day. In both types of studies, rats were treated with vehicle (−30 min), 3.2 mg/kg ketoprofen (−30 min), 3.2 mg/kg morphine (−30 min) or 32 mg/kg norbinaltorphimine (−24 hr) before subsequent treatment with vehicle, 0.56 mg/kg U69593 or dilute lactic acid (1.8 or 5.6% in water). All studies were approved by the Virginia Commonwealth University IACUC and were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Results: The acid noxious stimulus produced a concentration- and time-dependent depression of both ICSS and nucleus accumbens dopamine, and effects of the highest acid concentration (5.6%) in both assays were similar in magnitude to effects of 0.56 mg/kg U69593. Acid-induced depression of ICSS and nucleus accumbens dopamine was blocked by pretreatment with the analgesics ketoprofen and morphine, but not by the kappa antagonist norbinaltorphimine. Conversely, U69593-induced depression of ICSS and dopamine was blocked by norbinaltorphimine but not by ketoprofen; morphine produced intermediate effects. Neither ketoprofen nor norbinaltorphimine altered ICSS or dopamine levels when administered alone without acid. Morphine alone significantly increased basal dopamine, and produced biphasic effects on ICSS manifested as facilitation of low ICSS rates maintained by low brain stimulation frequencies and reduction in high ICSS rates maintained by high brain stimulation frequencies.

Conclusions: These results support a role for the mesolimbic dopamine system, but not of endogenous kappa opioid systems, in mediating pain-related depression of behavior in rats. Further research to investigate mechanisms of pain-related depression of behavior and mesolimbic dopamine levels may reveal new targets for analgesic drug development.

Keywords: pain, depression, intracranial self-stimulation, dopamine, kappa opioid

Disclosure: S. Negus, **Part 1:** Abbott, **Part 4:** Abbott; M. Leitl, **Part 1:** Employed as a staff biologist at Merck Research Laboratories from 2005-2011 before entering graduate school, **Part 2:** Employed as a staff biologist at Merck Research Laboratories from 2005-2011 before entering graduate school, **Part 3:** Employed as a staff biologist at Merck Research Laboratories from 2005-2011 before entering graduate school; M. Banks, **Part 1:** Purdue, Abbott, **Part 4:** Purdue, Abbott.

M53. Hippocampal Function in KCNH2-3.1 Transgenic Mice

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Background: Previously, our group identified the *KCNH2* gene, which codes for a human ether-a-go-go-related (ERG) potassium channel, as a risk gene for schizophrenia. Additionally, risk-associated alleles coincide with increased expression of the 3.1 isoform of *KCNH2* in the hippocampus. The *KCNH2*-3.1 isoform is missing a domain critical for the slow deactivation properties that characterize the ERG channels. Due to the change in channel structure, neurons that utilize ERG channels that contain the *KCNH2*-3.1 isoform produce a high-frequency, nonadaptive firing pattern. Patients carrying risk alleles associated with increased *KCNH2*-3.1 exhibit inefficient neural processing in temporal lobe-dependent behavioral tasks. In order to further probe the function of the *KCNH2*-3.1 isoform, we developed a transgenic mouse that expresses the protein. Previous work in our laboratory has shown that these *KCNH2*-3.1 transgenic mice are impaired in the hippocampal-dependent object location task. Specifically, the mice are unable to identify an object that has been displaced in a familiar contextual environment. These results suggest that *KCNH2*-3.1 expression affects the normal function of the hippocampus. The current experiments were designed to determine the extent of hippocampal dysfunction in the *KCNH2*-3.1 transgenic mice. We tested their performance in multiple cognitive domains subserved by the hippocampus.

Methods: We tested spatial recognition memory (Barnes maze) and hippocampal-dependent temporal processing (temporal order object recognition and trace fear conditioning) in *KCNH2*-3.1 transgenic mice and their wild-type littermates. The Barnes maze apparatus used in these experiments was similar to the one described by Holmes et al., (2002). Briefly, the procedure consisted of a training phase and a probe phase. The mice were given two training trials each day for a 10-day period. Following training, mice were tested in two probe trials given 24 hours and seven days following the end of training. The temporal order object recognition task was a modified version of the object recognition task used previously by our group (Papaleo et al., 2008). In the temporal order version, there are two sample phases (5 minutes each) in which identical copies of a novel object are presented. The objects are different between the sample phases and the two sample phases are separated by one hour. Three hours after the second sample phase, mice are presented with one copy of each of the two objects presented during the sample phases. During this test phase, mice have five minutes to freely explore both objects. Wild-type mice demonstrate temporal order recognition by spending more time exploring the object presented during the first sample phase. Intact hippocampal function is critical for normal performance in this task. The trace fear conditioning procedure produces an association between an aversive stimulus (foot shock) and a neutral stimulus (tone) so that presentation of the tone alone produces the fear response (freezing) previously only associated with the aversive stimulus. Trace conditioning is differentiated from conventional delay fear conditioning by the presence of a time interval (20 seconds) between presentation of the tone and the foot shock. Association between the tone and shock across the trace interval requires the hippocampus.

Results: In the Barnes maze, *KCNH2*-3.1 mice exhibit normal spatial reference learning and memory in the traditional version of the task. There were no differences between groups in the number of errors committed or time required to reach the goal box. *KCNH2*-3.1 mice also showed normal temporal order recognition in the test phase by spending more time exploring the object presented during sample 1. Additionally, novel object recognition and object exploration time were not different between genotypes. Finally, *KCNH2*-3.1 mice exhibited normal trace fear conditioning.

Conclusions: These experiments provide information on the extent of hippocampal dysfunction in *KCNH2*-3.1 transgenic mice. Previous work from our laboratory showed that *KCNH2*-3.1 mice exhibit severe impairment in the object location task. They are unable to identify a displaced object in a familiar context. The deficits in the hippocampal-dependent object location task were not due to deficits in object recognition, as *KCNH2*-3.1 mice perform normally in the object recognition task. Because of this result, we decided to investigate if *KCNH2*-3.1 mice display deficits

in other well-characterized hippocampal-dependent tasks. To this end, we focused on spatial reference memory and temporal encoding, two cognitive domains that require intact hippocampal functioning. *KCNH2*-3.1 mice performed normally in the traditional versions of the Barnes maze, temporal order object recognition, and trace fear conditioning. These data suggest that *KCNH2*-3.1 mice do not have global hippocampal function deficits as a result of their altered neuronal firing properties, but likely display a more specific phenotype. Future studies will focus on determining the parameters of hippocampal-dependent behaviors that lead to altered performance in *KCNH2*-3.1 mice.

Keywords: schizophrenia, hippocampus, potassium channel, learning and memory, animal model

Disclosure: G. Carr, Nothing to Disclose; A. Bebensee, Nothing to Disclose; R. Xun, Nothing to Disclose; O. Akhile, Nothing to Disclose; Q. Tian, Nothing to Disclose; J. Chen, Nothing to Disclose; F. Papaleo, Nothing to Disclose; D. Weinberger, Nothing to Disclose.

M54. Cocaine Craving and AMPA Receptor Plasticity: Modulation by Metabotropic Glutamate Receptors

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Background: After prolonged withdrawal from extended-access cocaine self-administration, Ca^{2+}-permeable AMPA receptors (CP-AMPARs) accumulate in the nucleus accumbens (NAc) and mediate the withdrawal-dependent intensification ("incubation") of cue-induced cocaine craving. Our goal is to understand mechanisms that normally restrict CP-AMPARs from NAc synapses in order to develop strategies to reverse or prevent CP-AMPAR accumulation during incubation and thus reduce craving. We have previously shown that *in vitro* activation of group I mGluRs in NAc slices obtained from "incubated" rats removes CP-AMPARs from NAc synapses in a mGluR1-dependent manner. The current studies assessed whether the same effect can be obtained with a positive allosteric modulator (PAM) of mGluR1 and whether systemic administration of this PAM can remove CP-AMPARs from NAc synapse and reduce cue-induced cocaine craving.

Methods: Patch-clamp recordings of medium spiny neurons in NAc slices obtained from "incubated rats" (extended-access cocaine self-administration and >40 days of withdrawal) were performed to assess whether *in vitro* administration of the mGluR1 PAM SYN119 (1 μM) removes CP-AMPARs from NAc synapses. To test the effect of *in vivo* SYN119 administration, rats received SYN119 (10 mg/kg, i.p.) prior to a test for cue-induced cocaine seeking; slices were then prepared for patch clamp recordings. Additional biochemical experiments were performed to assess the effects of *in vivo* administration of SYN119 on mGluR1 and GluA1 surface expression in the NAc.

Results: We found that acute bath application of SYN119 rapidly removes CP-AMPARs from NAc synapses. In addition, behavioral studies followed by patch clamp recordings showed that acute systemic administration of SYN119 reduces the expression of incubated cocaine-seeking and removes CP-AMPARs from NAc synapses. Based on these findings, we hypothesized that mGluR1 normally exerts inhibitory tone on CP-AMPAR levels in NAc synapses and that loss of mGluR1 tone during cocaine withdrawal enables CP-AMPAR accumulation. Indeed, we found that mGluR1 surface expression decreases slowly in the NAc during withdrawal, just preceding CP-AMPAR accumulation, and that restoring mGluR1 tone during this period by administering repeated, intermittent injections of SYN119 (10 mg/kg, i.p.) attenuates the incubation of cue-induced cocaine seeking. We then measured NAc surface levels of mGluR1 and GluA1 in these animals using biotinylation. Compared to vehicle controls, SYN119-treated rats showed an increase in mGluR1 surface levels, as well as a small but significant reduction in GluA1 surface levels.

Conclusions: These findings show that mGluR1 negatively regulates CP-AMPAR levels in the NAc. Acutely, mGluR1 PAM injection removes CP-AMPARs from NAc synapses and reduces cocaine craving. Furthermore, repeated mGluR1 PAM injections during a critical period of withdrawal oppose the decrease in surface mGluR1 that normally occurs, thus maintaining mGluR1-mediated inhibitory control over CP-AMPAR accumulation. Taken together, our results point to the potential utility of systemically active mGluR1 PAMs in the treatment of cue-induced cocaine craving and relapse in human addicts.

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Keywords: AMPARs, mGluR1, incubation, cocaine self-administration, positive allosteric modulators

Disclosure: J. Loweth, Nothing to Disclose; A. Scheyer, Nothing to Disclose; M. Milovanovic, Nothing to Disclose; X. Li, Nothing to Disclose; E. Flores-Barrera, Nothing to Disclose; M. Olive, Nothing to Disclose; K. Tseng, Nothing to Disclose; M. Wolf, Nothing to Disclose.

M55. Modulation of Decision Biases by the Lateral Habenula

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Background: Emerging evidence points to a key role for the lateral habenula (LHb) in mediating reward-related behavior, and dysfunction within this nucleus may contribute to aberrations in reward processing associated with a variety of psychiatric disorders. Involvement of the LHb in these processes stems in part from its ability to suppress activity of midbrain dopamine neurons. Much of the research on the LHb has focused on the relevance of its phasic signal, as transient increases and decreases in LHb activity have been observed in response to unexpected reward losses and gains, respectively. However, the manner in which activity within the LHb may regulate response selection where reward delivery is uncertain and that require cost/benefit appraisals of potential risks and rewards remains unclear.

Methods: The present study was designed to clarify how temporally-discrete stimulation or reversible inactivation of LHb alters decision making related to choice between certain and uncertain outcomes. Rats were well-trained on a probabilistic discounting task, requiring them to select between a small/certain reward (1 sucrose pellet) and a large/risky reward (4 pellets). The probability of receiving the large/risky reward was initially high (100-50%) and then decreased over blocks of discrete choice trials. In the first experiment, electrical stimulation of the LHb (20-80 pulses, 100 Hz, 200 uA) was administered to induce a temporally-discrete suppression of phasic dopamine activity that occurs during different rewarded outcomes (ie; immediately after a large/risky "win" or after smaller/certain reward delivery). In a second experiment, a separate group of rats received inactivation of the LHb via microinfusions of GABA agonists baclofen and muscimol (62.5 ng each in 0.25 ul) or saline on separate days.

Results: The first experiment revealed that phasic activation of LHb activity markedly influenced decision biases. Specifically, stimulation of the LHb only after risky "wins" decreased preference of the large/risky option. Conversely, stimulation only after receipt of the small/certain reward had the opposite effect, increasing risky choice. Similar effects were observed on non-stimulation probe trials, when the large or small reward was never delivered, which decreased/increased risky choice, respectively.

Importantly, LHb stimulation did not affect preference for larger vs. smaller reward when both were delivered with 100% certainty. In the inactivation experiment, rats displayed normal discounting of the large/risky option as the odds of obtaining the larger reward decreased across the session. This behavior was not altered following control infusions into the LHb, or inactivation of hippocampal or thalamic regions adjacent to the LHB nucleus. Surprisingly, and in stark contrast, infusion of GABA agonists into the LHb resulted in a pronounced flattening of the discounting curve, wherein rats showed no bias for either option during the first, 100% probability block, and this effect persisted over the duration of the session. Moreover, choice biases did not vary significantly across probability blocks, nor was choice of either option significantly different than chance (50%).
Conclusions: Collectively, these findings indicate that the LHb plays a critical and complex role in modifying risk/reward judgments and response selection. Temporally-specific phasic activation of the LHb after rewarded actions may "trick" the brain into thinking a received reward is less valuable which biases the direction of future choices, potentially via suppression of dopamine neuron activity. However, suppression of LHb firing reveals a more fundamental deficit in reward processing, characterized by a complete disruption of biases that emerge during evaluation of larger/uncertain versus smaller/certain rewards. This latter finding suggest that in choice situations, differential patterns of activity within the LHb associated with receipt or omission of different expected rewards serves to install choice biases towards options that may provide greater long-term utility.
Keywords: Lateral habenula, decision making, dopamine, reward
Disclosure: C. Stopper, Nothing to Disclose; S. Floresco, **Part 4:** I have received a grant from Pfizer to conduct contract work. The work in no way is related to the findings described in this abstract.

M56. Pavlovian Conditioned Approach to a Reward Cue Predicts Fear Incubation

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Background: A high proportion of patients with post-traumatic stress disorder (PTSD) also suffer from comorbid addiction, which suggests that some individuals may have a shared vulnerability to both disorders. The basic psychological processes underlying PTSD and addiction involve hyper-reactivity to trauma- or drug-related cues, respectively; the major theoretical difference between the two is in one sense simply the emotional valence of the unconditioned stimulus (US). An appetitive conditioning task called Pavlovian conditioned approach (PCA) can be used to classify animals based on whether they learn to approach and interact with a food predictive cue (sign-trackers; STs) or learn upon cue presentation to go to the location of impending food delivery (goal-trackers; GTs). Previous studies have shown that STs are more prone than GTs to attribute incentive salience to cocaine cues, and STs are more susceptible to cue-induced reinstatement of cocaine-seeking than GTs. Fear conditioning is a procedure that reliably produces a fear response to a neutral stimulus (e.g. a tone) after repeated pairings with an aversive stimulus (e.g. foot shock). Normally, a fear response develops quickly after as little as one tone-shock pairing, and very slowly decays over time. However, if the tone-shock pairing is repeated extensively, the fear response increases or "incubates" over time and becomes maximal ~30 days after conditioning, similar to the delayed-onset pattern of symptom development often seen in PTSD patients. We investigated whether PCA behavior, which predicts vulnerability to reinstatement of cocaine-seeking, also predicts fear incubation, which may be a marker for vulnerability to PTSD.
Methods: Animals first underwent 5 training sessions using a PCA procedure in which a retractable lever served as a cue or "sign" predicting delivery of a food reward. Each test session consisted of 25 trials, and each trial during a test session consisted of presentation of the illuminated lever (CS) into the chamber for 8 s. Retraction of the lever was immediately followed by the response-independent delivery of one food pellet (US) in the food receptacle. Rats were designated as ST vs. GT based on their response bias for the lever vs. food receptacle. For 10 days the same animals were given 10 tone-CS presentations (30 sec) per day, each of which coterminated with a foot shock US (1 s, 0.5 mA). The average intertrial interval (ITI) was 2 min. Movement was detected by displacement of a load-cell sensor, and freezing behavior was only scored if the rat was immobile for at least 1 second. Rats were designated as high- (HI) or low- (LO) conditioning based on a median split of their average freezing during the tone for all 100 tone-CS presentations. Animals then underwent operant conditioning such that they learned to nose poke at a steady rate of ~1 response/sec for a food pellet delivered on a variable-ratio schedule. The fear-conditioned tone-CS was then presented six times in the absence of shock during ongoing operant behavior to measure the change in rate of nose-poking either 3 days or 33 days after the last day of fear conditioning.
Results: There were no significant differences between STs and GTs in acquisition of the conditioned fear response (freezing) during training. Fear expression, as measured by conditioned suppression of nosepoking by the tone, was similar at 3 and 33 days after training in GTs. However, the fear response in STs was not apparent 3 days after training, and became significantly stronger after a 33-day incubation period. This fear incubation effect was particularly large among HI-conditioning animals who showed robust freezing responses during fear acquisition. The LO-conditioning animals with less freezing during training did not demonstrate any fear incubation among STs or GTs.
Conclusions: In this study, the fear incubation effect was only present among individuals who attributed excessive motivational salience to both appetitive and aversive conditioned cues. STs, thought to be more vulnerable to both cue- and drug-induced reinstatement models of addictive behavior, showed an abnormal PTSD-like fear response that was inhibited at first but increased over time. In contrast, GT behavior was consistent with a normal fear response that is most robust immediately after training and decays only very slowly over time. These results indicate that, while many factors likely contribute to the disproportionate co-occurrence of PTSD and substance abuse, one such factor may be a core psychological trait that biases some individuals to attribute excessive motivational significance to predictive cues, regardless of the emotional valence of those cues.
Keywords: addiction, post traumatic stress disorder, vulnerability, comorbidity, rat
Disclosure: J. Morrow, Nothing to Disclose; S. Maren, Nothing to Disclose; T. Robinson, Nothing to Disclose.

M57. Hyperactivity and Increased Sociability in Mice Lacking Fibroblast Growth Factor Receptor 2 in GFAP+ Cells During Critical Early Postnatal Period

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Background: Our laboratory has shown that Fibroblast Growth Factor Receptor (FGFR) knock-out mice show significant alterations in locomotor activity, exploratory activity and learning and memory, a behavioral profile that resembles neuropsychiatric disorders arising in childhood. FGF signaling has been shown to be essential for the proper embryonic development of the neocortex—early elimination of FGF receptors (FGFRs) results in cortical and hippocampal volume reduction and decreased excitatory and

inhibitory neuron numbers. However, the role of FGF receptors in significant early postnatal events like gliogenesis and synaptic outgrowth has been not yet explored. We investigated the role of FGF signaling, particularly through FGF receptor 2 (FGFR2), during the early postnatal period of development to determine the timing of FGF's role in establishing the neuronal circuits that regulates locomotion, working memory, social interaction and exploration, critical components in disorders like ADHD.

Methods: Mice had either conditional or inducible forms of a hGFAP Cre system mediating the knock out of a floxed *fgfr2* gene. Conditional FGFR2KO animals have widespread inactivation of *fgfr2* in both telencephalic neurons and glia due to early activation of Cre in radial glia of the dorsal telencephalon at embryonic day 13.5 (E13.5). Inducible FGFR2KO mice have inactivation of *fgfr2* restricted to postnatal astrocytes due to tamoxifen-inducible Cre activation in GFAP+ cells of the dorsal telencephalon, through maternal injections from postnatal day 1 (P1) to P6 (perinatal induction) or at P56 to P60 (adult induction). FGFR2 expression was evaluated with quantitative PCR. Behavior testing was completed at approximately age 3 months in an open field paradigm, a 3-chamber social approach paradigm, and spontaneous alternation in a Y-maze.

Results: Both conditional FGFR2KO mice and perinatally-induced FGFR2KO mice show increased locomotor activity and increased exploration of the center in an open field paradigm compared to Cre- controls. In contrast, adult-induced FGFR2KO mice showed no change in locomotor activity, but did show increased center exploration. Likewise, both conditional FGFR2KO and perinatally-induced FGFR2KO mice showed deficits in spontaneous alternation and increased social approach while adult-induced FGFR2KO mice were similar to Cre- controls in these tasks. Despite significant behavioral alterations evaluated in adulthood in perinatally-induced FGFR2 KO mice, only a temporary deficit in *fgfr2* expression was found following induction, without any loss of *fgfr2* expression after the age of 2-3 weeks.

Conclusions: FGFR2 plays a significant role in early postnatal development of the dorsal telencephalon, as even temporary reductions in receptor level during this period result in significant alterations in multiple domains including sociability, locomotion/exploration and working memory. These findings may highlight a role for FGFR2 in GFAP+ cells during the first week of mouse life. Multiple roles for GFAP+ cells have been shown for normal neuronal structure and function which could be ongoing during this time period including gliogenesis, regulation of lactate metabolism, and the regulation of glutamate uptake and metabolism. FGFR2 deficits in GFAP+ cells may implicate one or more of these functions in neuropsychiatric disorders of childhood.

Keywords: Fibroblast growth factor, glia, social behavior, locomotor activity

Disclosure: H. Stevens, **Part 4:** APIRE-Wyeth Research Fellowship in 2010; F. Vaccarino, Nothing to Disclose.

M58. Adolescent Cannabinoid Exposure and Schizophrenia-like Deficits

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Background: Cannabis is the most commonly abused illicit drug in the United States with about 60% of the 2.4 million marijuana initiates in 2010 being less than 18 years of age. Daily marijuana use is now at a 30-year peak level among high school seniors. This is of particular health concern given the large body of literature that shows an association between adolescent cannabis use and adult onset of psychosis. Schizophrenia did not develop days or weeks after cannabis use, but years later, suggesting that cannabis use during a critical period of brain maturation may lead to long-term effects. These human studies demonstrate associations but do not demonstrate causality.

Methods: We examined the causal relationship between developmental cannabinoid administration and long term behavioral and molecular alterations in mice. Mice were administered either WIN 55,212-2 (WIN), a cannabinoid receptor1 (CB1) agonist or vehicle (Veh) during adolescence (PND 30-35) or early adulthood (PND 63-70). Behavioral testing was conducted after PND 120 followed by biochemical assays.

Results: Adolescent cannabinoid treatment (ACU) leads to deficits in prepulse inhibition and fear conditioning in adulthood. mGluR5, a receptorcritically involved in fear conditioning and endocannabinoid (eCB) signaling, is significantly reduced in the ACU mouse hippocampus (Veh 5.5 ± 0.7; ACU 3.15 ± 0.34 units; $p = 0.007$). Next, we examined expression profiles of genes involved in eCB synthesis (DGL) and uptake (MGL, FAAH) in the experimental mice. We find evidence of increased MGL (Veh 2.0 ± 0.28; ACU 4.58 ± 0.62 units; $p = 0.001$) and FAAH (Veh 2.82 ± 0.56; ACU 6.7 ± 1.33 units; $p = 0.014$) in ACU mice, reflecting increases in eCB uptake and degradation.

Conclusions: These data suggest that administration of cannabinoids during adolescence leads to a behavioral phenotype associated with a rodentmodel of schizophrenia, as indexed by alterations in sensorimotor gating and hippocampal dependent learning and memory deficits. Further, these deficits are associated with a reduction in hippocampal mGluR5 and a sustained change in eCB turnover, suggesting reduced endocannabinoid signaling in the ACU hippocampus. These data provide evidence suggesting that cannabis use during adolescence may be a causal factor in the development of certain features of schizophrenia and may suggest the candidacy of mGluR5 as a potential therapeutic target for ACU-induced schizophrenia.

Keywords: hippocampus, adolescence, cannabis, endocannabinoid, mGluR5

Disclosure: S. Ghose, Nothing to Disclose; K. Gleason, Nothing to Disclose; A. Shukla, Nothing to Disclose; S. Birnbaum, Nothing to Disclose.

M59. Social Interaction Familiarization, a Valid Preclinical Model of Social Processing and Behavioral Therapy for Anxiety

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Background: Many forms of anxiety, including phobias and social anxiety disorder (SAD), are responsive to therapies where repeated exposure and familiarity to the anxiogenic object eventually alleviates the anxiety, a process that can be enhanced by d-cycloserine. Despite the success of these exposure therapies, little is known about their mechanisms and neural circuitry. Elucidation of these neural circuits and mechanisms has been in part hindered by lack of valid preclinical, animal models. Recently, it was demonstrated that in rats, social familiarity alleviates anxiety-like behavior in the social interaction (SI) test, a procedure referred to as the Social Interaction Familiarization (SIF). SIF consists of a 5 min SI repeated daily with the experimental rat exposed to the same partner rat on each subsequent test day. Here we characterize and validate the SIF test as a model for social processing and exposure therapy for SAD.

Methods: Exp. 1: Using bright light (bl, turned on at start of SI test) as an ethological anxiogenic challenge, anxiety-like responses were assessed using the SI test. Rats were subsequently exposed (only during SI testing) to the same "familiar" partner rat, or to an unfamiliar partner rat for additional 5 SI test performed daily, under bl or dl. Exp. 2: Using similar procedure, generalization of anxiolytic responses were tested using bl or restraint stress as anxiogenic cues. Exp. 3: Rats were tested in the SIF paradigm in the

presence or absence of d-cycloserine (10 mg/Kg sc) 30 min prior to the 2nd exposure to the familiar partner rat and on each subsequent SI session. Exp. 4: The role of the medial ventral prefrontal cortex (mvPFC) in the social interaction familiarity effect was investigated using similar paradigm as above, but prior to each familiarity session the mvPFC was inhibited by intracranial injection of the GABA A rec agonist muscimol injected bilaterally into the mvPFC.
Results: Exp. 1: The anxiety-like behavior induced by bl was diminished as familiarity to the partner rat increased. SI times returned to baseline (dl) levels and, compared to 1st day of bl exposure, were significantly increased on days 5 and 6. In control rats, bl-induced decreases in SI time remained constant on subsequent days of SI testing when an unfamiliar partner was introduced each testing session, suggesting that the reduction in anxiety-like responses were related to social and not contextual familiarity. Additionally, SI time was not increased with familiarity to a partner rat in the absence of an anxiogenic challenge (under dl conditions), suggesting the increase in SI time was likely a selective reduction in bl-induced anxiety-like response and not a generic non-specific increase of SI following repeated exposure. Exp 2: Similarly, social familiarity also produced anxiolytic-like responses to restraint stress, however, familiarity did not alter anxiogenic-like responses to restraint stress when the familiarity was paired with bl. Exp. 3: Addition of d-cycloserine into the SIF test reduced the number of familiarity pairings necessary to significantly increase SI time from first BL exposure. Exp. 4: Inhibiting the mvPFC blocked the anxiolytic-like effect induced by social familiarity.
Conclusions: These data validate the SIF test as a preclinical model for investigating regulation of anxiety to social cues that has face, predictive (d-cycloserine results) and construct validity (mvPFC results).
Keywords: social anxiety exposure therapy
Disclosure: W. Truitt, Nothing to Disclose; E. Lungwitz, Nothing to Disclose; A. Dietrich, Nothing to Disclose; P. Minick, Nothing to Disclose; A. Shekhar, Nothing to Disclose.

M60. Alarm Pheromone Processing Areas are Involved in the Intergenerational Social Transfer of Emotional Trauma

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Background: A number of studies suggest that psychological trauma can be transmitted to subsequent generations and potentiate the emergence of mental disorders, such as depression, PTSD and specific phobias (Cameron et al., 2005; Yehuda et al., 1998; de Rosnay et al. 2006). We have recently proposed a rat model of a transgenerationally transmitted trauma (Debiec and Sullivan, ACNP 2011). Using 2-DG autoradiographic imaging, we have shown that the lateral nucleus of the amygdala, a key structure underlying associative fear learning, is involved in the transfer of fear responses from mothers to infants (Debiec and Sullivan, ACNP 2011). We have demonstrated that in our paradigm, the social transmission of fear from mothers to their pups is mediated by maternal alarm odor (Debiec and Sullivan, ACNP 2011). Here we ask whether brain structures which are known to process alarm pheromones are also involved in the acquisition of socially transmitted fear.
Methods: Female rats that had undergone olfactory fear conditioning training were re-exposed to their conditioning stimulus (CS) in the presence of the pups ("Paired-CS"; $n = 7$). Controlled groups included pups exposed to mothers that had been previously conditioned but were not re-exposed to the CS ('Paired-No CS"; $n = 4$) and pups exposed to mothers that had been previously exposed to unpaired presentations of CS odor and electric shock ("Unpaired-CS"; $n = 6$). All pups were injected with ^{14}C S 2-DG prior to their exposure in order to assess the neural changes during acquisition. Following exposure to their mothers, 2-DG reuptake in pups' brains was assessed.
Results: Statistical analysis revealed that "Unpaired-CS" group exhibited decrease of 2-DG uptake in the granular part of the accessory olfactory bulb (AOB) as compared to two other groups [ANOVA, $F(2, 14) = 6.089$; $p < 0.02$; *post hoc* Newman-Keuls Multiple Comparison Test $p < 0.05$]. "Paired-CS" group showed significant increase of 2-DG uptake in necklace glomeruli (NG) [ANOVA, $F(2, 14) = 8.438$; $p < 0.004$; *post hoc* Newman-Keuls Multiple Comparison Test $p < 0.05$].
Conclusions: Our data demonstrate that the acquisition of socially transmitted fear in rat pups involves the AOB and the NG which are both involved in processing of intraspecific chemical alarm signaling (Chamero *et al*., 2012; Luo, 2008). Interestingly, there was no difference in 2-DG uptake in the AOB between the "Paired-CS" and "Paired-No CS" groups suggesting that the history of trauma (fear conditioning) predicts fear and the AOB activation. In contrast, the 2-DG uptake in the NG was significantly increased as compared to two other groups. This pattern of findings suggests that the NG activity underlies acquisition of socially transmitted fear. This research was supported by grants NIDCD DC009910 and NIMH MH091451 to RMS and NARSAD Young Investigator Award from the Brain & Behavior Research Foundation to JD.
Keywords: Fear, trauma, social, memory, pheromone
Disclosure: J. Debiec, Nothing to Disclose; R. Sullivan, Nothing to Disclose.

M61. Adolescent Stressors to Epigenetic Modulation in Dopaminergic Neurons via Glucocorticoids: A Novel Model for Psychotic Depression

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Background: Human behavior in adulthood is crucially influenced by various environmental conditions during childhood and adolescence. Nonetheless, individual responses to such environmental factors vary, mainly because of different genetic predispositions among individuals. These gene-environmental interactions may also underlie a variety of neuropsychiatric disorders. Elucidation of the underlying mechanisms and mediators should help development of a means to intervene in such disorders, including prophylactic environmental readjustment.
Methods: A genetic model with isolation stress was examined by behavioral assays and neurochemical assessments. A glucocorticoid receptor antagonist RU38486 (mifepristone) was used. The nuclei of mesocortical and mesolimbic dopaminergic neurons in ventral tegmental area were enriched by labeling with fluorescent retrograde beads and fluorescence-activated cell sorting in a projection-specific manner. Epigenetic modification of the gene for tyrosine hydroxylase was examined by bisulfite sequencing.
Results: We exposed a genetic model (DISC1 mutant mice) to 3-week isolation stress during adolescence (from 5 to 8 weeks of age) and observed behavioral deficits (prepulse inhibition, forced swim test, and locomotor activity) and neurochemical changes associated with dopamine in adulthood. Two distinct dopaminergic projections (mesocortical and mesolimbic) originating from the ventral tegmental area was differentially affected in this model. A mild isolation stress with the genetic risk affected only mesocortical, but not mesolimbic, projection of dopaminergic neurons in which DNA hypermethylation of the tyrosine hydroxylase gene was elicited. The epigenetic alternations were long-lasting, evident in adult animals even if they were maintained in the normal group housing until 20 weeks after the transient adolescent isolation. These molecular, neurochemical, and

behavioral deficits in this model were normalized by an administration of a glucocorticoid receptor antagonist RU38486. Given that behavioral deficits may be relevant to endophenotypes of psychotic depression and that RU38486 uniquely benefits this condition, this model of gene-environmental interaction may be a promising tool to study psychotic depression.
Conclusions: This study shows a novel link between adolescent stressors and epigenetic controls in dopaminergic neurons via glucocorticoids, which addresses a central question of neurobiology of how adult behavior patterns are formed by a combination of genetic factors and environmental stressors. Interestingly, the epigenetic modifications by the primary stressor are maintained for long (the isolation stress during adolescence leads to a long-lasting change in adulthood). The present study also offers an innovative mouse model for psychotic depression, a common and debilitating psychiatric disease. The availability of a preclinical model would allow us to study underlying pathological mechanisms, including those in the premorbid and prodromal stages, and explore novel therapeutic strategies. Such a model could provide a good template not only for screening compounds with better efficacy and fewer side effects, but also for prophylactic environmental readjustment, which is crucially important in clinical psychiatry.
Keywords: adolescent stress, dopamine, glucocorticoid, epigenetics, psychotic depression
Disclosure: M. Niwa, Nothing to Disclose; A. Sawa, **Part 1:** Research Funding: Astellas, Takeda, Tanabe-Mitsubishi, Dainippon-Sumitomo, Consultant: Pfizer, Asubio, Sucampo, Eli Lilly, Taisho, Amgen, Collaboration: Pfizer, Afraxis, Sanofi-Aventis, Astrazeneca, Johnson and Johnson.

M62. Enhanced Nicotine Self-administration in the Neonatal Ventral Hippocampal Lesion Rat Model of Schizophrenia without Nicotine Reversal of Spatial Working Memory Deficits

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Background: Greater than 80% of people suffering with schizophrenia also have nicotine dependence, representing a 4-fold increase in prevalence over the general population. Despite increased morbidity and mortality, psychiatric treatment non-compliance and financial destitution associated with smoking in schizophrenia, prevailing theories and clinical research efforts exploring this comorbidity have focused on the hypothesis that these patients smoke so much to receive beneficial-medicinal effects of nicotine, particularly for cognitive deficits in the disorder. We employed a widely studied animal model of schizophrenia—neonatal ventral hippocampal lesions (NVHL) in rats—to examine the extent to which this comorbidity may be better explained from an addiction vulnerability vs. self-medication framework.
Methods: In experiment 1, NVHL and SHAM-operated control rats ($N = 45$) were compared in sensitization to nicotine (vs. saline injections) during adolescence (nicotine 0.5 mg/kg/day; Post-natal Days (PD35-44)) followed in adulthood ($PD > 60$) by i.v. nicotine self-administration (0.015 µg/ injection; 2 h sessions) where the main effects of lesion status and adolescent nicotine history were assessed across acquisition, dose response testing and drug-seeking during extinction. Experiment 2 used separate groups of NVHL vs. SHAM rats ($N = 37$) to examine the effects of daily nicotine injections in adulthood (0.5 mg/kg/day; 30 minutes before behavioral testing) on spatial learning and memory as tested on the radial arm maze (RAM).
Results: In experiment 1, although nicotine injections produced robust levels of behavioral sensitization during adolescence, NVHLs trended toward, but did not show significantly increased levels of sensitization compared to SHAMs, in contrast to prior findings where NVHL rats tested in adulthood show robustly increased nicotine sensitization compared to SHAMs. NVHL rats went on in adulthood to show significantly increased levels of nicotine self-administration during acquisition and over the entire course of i.v. nicotine exposure. Adolescent nicotine experience did not alter acquisition but changed the sensitivity to nicotine in the dose response testing, so that adolescent exposed rats consumed more nicotine at the highest dose self-administered. During extinction, NVHL rats demonstrated greater nicotine-paired lever responding. In experiment 2, deficits in RAM learning and memory due to NVHLs were not corrected by nicotine injections, and in fact, cessation of nicotine injections during RAM testing produced detrimental consequences for cognition in both NVHL and SHAM rats alike.
Conclusions: In an extensively studied animal model of schizophrenia known for its utility in parsimoniously modeling core symptoms and many of the pharmacological, developmental, and neurobiological features of human schizophrenia, we observed an enhancement of the addictive properties of nicotine without evidence for nicotine-induced enhancement in cognition. These findings call into question the validity of self-medication –based explanations for smoking in schizophrenia. A deeper understanding of the neural mechanisms that may drive nicotine addiction vulnerability in schizophrenia as illuminated by novel animal modeling and clinical approaches are needed to define more effective preventative and treatment strategies for nicotine addiction comorbidity beyond what can be achieved based on traditionally-held claims that nicotine is therapeutic for schizophrenia.
Keywords: Nicotine, addiction, ventral hippocampus, schizophrenia, self-medication
Disclosure: S. Berg, Nothing to Disclose; A. Sentir, Nothing to Disclose; B. Cooley, Nothing to Disclose; R. Chambers, Nothing to Disclose.

M63. Convergence of Medial and Orbital Prefrontal Cortical Fibers in the Ventral Striatum Mediate DBS-enhancement of Fear Extinction

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Background: Deep brain stimulation (DBS) of the VC/VS (ventral capsule/ventral striatum) in humans reduces symptoms of intractable obsessive-compulsive disorder (OCD), however the mechanisms of action are unknown. A prominent feature observed in most OCD patients is repetitive avoidance behaviors to perceived threats. The persistent avoidance in OCD suggests a deficit in circuits that regulate fear extinction. Given the strong parallels between fear circuits in rodents and humans (Milad & Quirk, 2012), well-characterized rodent models of fear extinction can shed light on DBS mechanisms in OCD and related illnesses that feature pathological anxiety. Using a rat model of fear extinction, we recently reported that DBS of a specific zone of the dorsal portion of ventral striatum (just above the anterior commissure) facilitated fear extinction and induced plasticity in cortico-amygdalar circuits, whereas DBS of more ventral sites impaired fear extinction and did not induce plasticity (Rodriguez-Romaguera et al., 2012). This suggests that clinical DBS may act, at least in part, by facilitating extinction of fear. The marked difference between DBS in the dorsal and ventral portions of the ventral striatum suggests that these regions contain distinct sets of descending cortical myelinated fibers that are modulated by DBS. It has been well characterized that within medial PFC, the infralimbic cortex (IL) is important for fear extinction whereas the prelimbic cortex (PL) is important for fear expression. We hypothesize that an anatomical dissociation within IL and PL

fibers in the ventral striatum could explain the opposite effects of DBS on fear extinction.
Methods: To test this hypothesis, we injected the retrograde tracer *wheat germ agglutinin* into the dorsal (VSd, n = 3) or ventral (VSv, n = 2) portions of the ventral striatum that are associated with enhanced or impaired fear extinction respectively. The goal was to determine the relative contribution of cortical inputs to each area from different prefrontal regions. Using stereological techniques we analyzed the number of labeled cell in the medial (mPFC), orbital (OFC) and anterior insular (AI) prefrontal cortices following each injection. The mPFC was subdivided into the cingulate (Cg1), prelimbic (PL) and infralimbic (IL) cortices, the OFC into the medial (MO) and ventrolateral (VLO) orbitofrontal cortices and the AI into the ventral (AIv) and dorsal (AId) anterior insular cortices. We used the proportion of cells within a structure (% of total cells counted) to analyze the distribution of cortical cell labeling for each injection site, and the cell density (neurons/mm^2) to compare the differences between injection sites and between cortical structures.
Results: The VSd site showed a largest proportion of labeled cells in PL (36%), VLO (30%) and MO (19%). In contrast, the VSv site showed a largest proportion in PL (39%) and IL (20%). Cg1, AIv and AId had similar proportions from both VSd and VSv ($\leq$ 8%). When comparing cell density between injection sites, we found that Cg1, AIv, AId and PL were equal in both VSd and VSv. However, the VSd site had a higher cell density in both MO (VSd: 43; VSv: 13; $p = 0.04$) and VLO (VSd: 13; VSv: 4; $p = 0.03$) compared to VSv. In contrast, the VSv site had a higher cell density in IL (VSd: 8; VSv: 43; $p = 0.006$). Further analysis, comparing cell density between cortical regions within an injection site showed that the VSd had the highest cell density in PL as compared to IL (PL: 22; IL: 8; $p = 0.04$), along with a high density of cells in MO as compared to VLO (MO: 43; VLO: 13; $p = 0.01$). Contrary to VSd, the VSv had the highest cell density in IL as compared to PL (IL: 43; PL: 15; $p = 0.04$). In insular cortex, AIv and AId had equal cell densities across both injection sites. In summary, the VSd site had a higher proportion of projecting neurons from PL, VLO and MO, whereas the VSv site had a higher proportion from PL and IL. The VSd site is also characterized by having a stronger projection from PL as compared to IL, while the VSv site is characterized by having a stronger projection from IL as compared to PL.
Conclusions: Previous studies have demonstrated the topographic organization of cortico- striatal projections. However, we show here that there is considerable convergence of inputs from distinct cortical areas into VS regions that differentially modulate fear extinction. However, the proportion of cortical inputs differs within these VS sites. Our data suggests that the DBS-enhancement of extinction with stimulation in VSd may require coordinated changes within a larger medial-orbital PFC network (PL, MO and VLO). On the other hand, the DBS-impairment of extinction with stimulation in VSv may be due to a more constraint medial PFC network (PL and IL). In accordance with previous studies suggesting that striatal DBS inhibits activity of cortical regions (McCracken and Grace, 2007; 2009), our anatomical findings suggest that DBS in VSv preferentially inhibits IL over PL (impairing extinction) and that DBS in VSd preferentially inhibits PL over IL (reducing fear and enhancing extinction). Thus, the variation between the magnitude of IL and PL inputs to the VSd and VSv may help explain the differences in behavioral effects of DBS on fear extinction.
Keywords: ventral striatum, prelimbic, infralimbic, orbitofrontal, deep brain stimulation
Disclosure: J. Rodriguez-Romaguera, Nothing to Disclose; F. Do Monte, Nothing to Disclose; Y. Tanimura, Nothing to Disclose; G. Quirk, Nothing to Disclose; S. Haber, **Part 1:** Pfizer and Medtronics.

M64. Early Exposure to Antidepressants Does Not Recapitulate Constitutive Serotonin Transporter Deficiency

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Background: Administration of serotonin transporter inhibiting antidepressants (SSRIs) during a critical early postnatal period in rodents is postulated to reproduce changes in behavior arising from constitutive reductions in serotonin transporter (SERT) expression. Both animal models have medical significance related to neonatal exposure to SSRIs and differential SERT expression associated with human *Sert* gene polymorphisms, respectively.
Methods: We investigated the effects of postnatal administration of escitalopram (S-CIT) or fluoxetine *vs* constitutive SERT deficiency in mice on emotion-related behaviors, presynaptic 5-HT1A receptor expression and function, and extracellular serotonin levels in adolescence and late into adulthood. SSRIs were administered daily during postnatal days 5-21. Behavior was assessed in the elevated plus maze, open field, forced swim test, and sucrose preference test. Thermic responses to the 5-HT1A receptor agonist 8-OH-DPAT were investigated. *In vivo* microdialysis and zero-net-flux analysis were used to evaluate changes in extracellular serotonin levels.
Results: Increased anxiety-related behavior, which is highly characteristic of SERT-deficient mice and humans with low-expression *Sert* alleles, was notably absent or potentiated in mice postnatally exposed to SSRIs. Furthermore, whereas 5-HT1A-mediated decreases in body temperature were attenuated in SERT-deficient mice, SSRI-treated mice showed pronounced hypothermia after 8-OH-DPAT. Moreover, postnatal treatment with S-CIT resulted in 5-HT1A autoreceptor *hyper*sensitivity. Extracellular serotonin levels have been shown to be elevated in mice with constitutive reductions in SERT, however, mice exposed to SSRIs during early postnatal development showed reduced extracellular serotonin levels as adults.
Conclusions: Transient *vs* constitutive SERT deficiency produces opposing and long-lasting changes in regulation of extracellular serotonin and 5-HT1A autoreceptor function evident as early as adolescence. Persistent changes in presynaptic serotonergic circuitry are hypothesized to contribute to differential emotional phenotypes associated with these two models. Ongoing work is aimed at investigating mechanisms of differential serotonin and 5-HT1A regulation. These findings have important implications for antidepressant use during pregnancy and neonatal life in humans, which corresponds to the early postnatal period in rodents. They further clarify genetic influences associated with differential SERT expression regarding effects on behavior and underlying neurotransmission.
Keywords: Developmnet Anxiety 5-HT1A Behavior Microdialysis
Disclosure: S. Altieri, Nothing to Disclose; H. Yang, Nothing to Disclose; H. O'Brien, Nothing to Disclose; J. Hensler, Nothing to Disclose; A. Andrews, Nothing to Disclose.

M65. Serotonin Transporter Genotype Modulates HPA Axis Output During Stress: Effect of Stress, Dexamethasone Test and ACTH Challenge

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Background: Studies show that the HPA Axis is dysregulated in depression. Some studies have suggested that variation in the serotonin transporter genotype (*5HTT)* may be associated with both risk for depression and psychopathological HPA Axis responsiveness. Previous studies of rhesus macaques modeling human psychopathology have assessed the effect of the serotonin

transporter (*rh5HTT)* on levels of cortisol in stressed subjects. These studies show that that under conditions of stress, heterozygous females (L/s) reared under adversity exhibit high levels of cortisol. Studies have not to our knowledge, however, assessed the potential additive effect on the cortisol response in a large number of macaque subjects homozygous for the serotonin transporter short allele (s/s). Moreover, little is known about the level of the central or peripheral nervous system at which the *5HTT* genotype acts to modulate the cortisol response.

Methods: This study assesses a large number of subjects homozygous and heterozygous for the *rh5HTT* short and long alleles A.) during stress, B.) following a dexamethasone suppression test, and C.) following an ACTH challenge. For this study, we tested 191 infant rhesus macaques (107 females; 84 males), obtaining 2 blood plasma samples during the stress of a separation from their mothers. Then on the following day, we obtained a blood sample following a dexamethasone test, and later that day we obtained a blood sample after an ACTH challenge test. Subjects ranged in age between 90 and 128 days, with a mean age of 107 days. Preliminary analyses showed no effect for gender on plasma cortisol in this age of subjects, and given that the two stress samples were highly correlated, we used the mean value for the two stress samples as one dependent variable, with the dexamethasone, and the ACTH values being the other two dependent measures in a mixed design ANOVA. Age was used as covariate and a two-way analysis of covariance was performed with genotype being the between groups variable (L/L = 119, L/S = 60, S/S = 12).

Results: With varying days of age statistically controlled, subjects homozygous for the short allele had significantly higher levels of cortisol across all test conditions ($p < .04$), when compared to those homozygous for the long allele, or those heterozygous with L/s alleles. Subsequent analyses showed a high correlation for individual cortisol levels across the three different tests.

Conclusions: These data suggest that subjects homozygous for the short allele are more likely to show dysregulated cortisol levels in response to stress, with no difference between the L/L and L/s genotypes. Given the correlation in individual responses of the HPA Axis across the different tests, our data suggest that the effect of the *5HTT* genotype is likely at the central level, possibly in the CRH system. Our data suggest that under conditions of stress, the serotonin transporter may modulate both depression and HPA Axis psychopathology.

Keywords: HPA Axis Serotonin transporter genotype Cortisol Stress Depression

Disclosure: J. Higley, Nothing to Disclose; A. Sorenson, Nothing to Disclose; J. Capitanio, Nothing to Disclose; S. Mendosa, Nothing to Disclose.

M66. Aberrant Light Impairs Mood and Learning through Melanopsin-expressing Neurons

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Background: Many biological functions oscillate over the course of a day, and the precise timing of these rhythms depends on synchronization to the solar cycle. Variation in day length, shift-work, and transmeridian travel, which disrupt normal exposure to the daily light/dark cycle, can lead to changes in mood and cognition. Several studies have shown that sleep deprivation and circadian disruption underlie the changes in mood and cognitive function associated with irregular light schedules. However, little is known regarding the ability of light to directly influence these functions. The goal of our study was to determine the influence of light on mood and cognitive functions in the context of normal circadian rhythms and sleep. Additionally, we sought to identify the retinal mechanism underlying the ability of light to influence these functions. We focused on a specialized ganglion cell population that are intrinsically photosensitive (ipRGCs) due to expression of the photopigment, melanopsin. In addition to their role in regulation of circadian rhythms and sleep, these cells project to limbic regions indicating a possible role in the regulation of mood and cognitive function.

Methods: To gain a better understanding of how light influences mood and cognitive functions, we housed mice in an environment that provided exposure to light pulses throughout the circadian cycle. We used EEG/EMG recording to assess sleep and examined the circadian system by measuring rhythms in general activity, core body temperature, as well as circadian gene expression. Using a combination of behavioral, biochemical, and electrophysiological techniques, we assessed mood related behaviors as well as learning and memory in mice housed under this light environment. We examined mice lacking ipRGCs housed under this disruptive light environment in order to determine the underlying retinal circuit responsible to conveying this light information.

Results: Mice exposed to this aberrant light cycle maintained intact circadian rhythms and normal sleep; however, they elicited increased depression related behavior as well as hippocampal learning deficits with corresponding physiological changes such as elevated corticosterone and deficient long-term potentiation. Chronic administration of the antidepressant fluoxetine rescued the increased depression related behavior and learning deficits induced by this light cycle. Furthermore, this treatment restored normal corticosterone levels and long-term potentation. Mice lacking ipRGCs were unaffected by exposure to light pulses throughout the circadian cycle showing that these cells are responsible for conveying light information to areas of the brain that control learning and mood.

Conclusions: Changes in the light environment can lead to disruptions in circadian rhythms and sleep and also cause changes in mood and learning deficits. The contribution of light to the changes observed in mood and cognitive function has been previously considered to occur through the modulation of sleep and circadian rhythms. Our findings present an additional pathway whereby light can more directly impact mood and cognitive functions without first disrupting sleep and circadian rhythms. Additionally, we present a novel role for ipRGCs in conveying light information to influence these functions.

Keywords: depression, learning and memory, light, circadian rhythms, sleep

Disclosure: T. LeGates, Nothing to Disclose; C. Altimus, Nothing to Disclose; H. Wang, Nothing to Disclose; S. Yang, Nothing to Disclose; A. Kirkwood, Nothing to Disclose; T. Weber, Nothing to Disclose; S. Hattar, Nothing to Disclose.

M67. Traumatic Stress Reactivity Facilitates Excessive Alcohol Drinking and Prefrontal Cortex-Amygdala Synchronicity

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Background: PTSD and alcoholism exhibit a high degree of co-morbidity in humans and have partially overlapping symptomatic profiles. The aim of this study was to examine changes in neuronal activation profiles in relation to stress-induced behavioral changes.

Methods: Male Wistar rats were trained to respond for 10% w/v ethanol vs. water on a continuous reinforcement schedule in a two-lever operant situation. Rats were then exposed to two contexts that differed on two sensory modalities (visual & tactile stimuli). Rats were divided into two groups (conditioned & controls) matched for chamber preference and ethanol responding. Conditioned rats were exposed to neutral odor in non-preferred context and predator odor (bobcat urine) in preferred context, then tested 24 hrs later for conditioned place avoidance (CPA). Conditioned rats were divided into two groups (avoiders & non-avoiders) based

on avoidance of predator odor-paired context relative to baseline (avoiders defined by ≥10-s decrease in time spent in predator odor-paired context during 5-min test). Rats were tested for operant ethanol responding and persistence of avoidance behavior (6 wks post-conditioning). Rats were re-exposed to predator odor-paired context, sacrificed, and brains removed for Western blot analysis of extracellular signal-related kinase (ERK) and phosphorylated ERK (pERK) expression.
Results: Relative to Non-Avoiders and Controls, Avoiders exhibited consistently higher avoidance of the predator-paired chamber, and higher operant alcohol responding. The two major findings from neuronal activation experiments were that: [1] relative to unstressed control animals, Avoiders exhibited higher stress context-induced ERK phosphorylation in vmPFC compared to Non-Avoiders that was significantly correlated with avoidance of the stress-paired context, and [2] Avoiders exhibited much stronger synchronicity between dmPFC and BLA activation, and activation profiles in both dmPFC and BLA correlated with post-conditioning increases in alcohol drinking in Avoiders.
Conclusions: This study presents a novel animal model for assessing the effect of a traumatic stressor and the maladaptive response to that stressor on subsequent alcohol drinking. This model also describes unique neuronal activation patterns that differ between animals according to traumatic stress response and are predictive of alcohol-drinking behavior.
Keywords: PTSD, Alcoholism, pERK, Prefrontal Cortex, Amygdala
Disclosure: N. Gilpin, Nothing to Disclose; S. Edwards, Nothing to Disclose; G. Koob, **Part 1:** Addex Pharmaceuticals, Alkermes, Arkeo Pharmaceuticals, Casa Palmera, Embera NeuroTherapeutics, GlaxoSmithKline, Lilly, Psychogenics.

M68. Context-dependent Neuronal Ensembles in the Amygdala, Prelimbic Area and Ventral Hippocampus after Fear Extinction in Rats

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Background: It has been repeatedly shown that after extinction, traumatic fear can persist indefinitely across contextual shifts. Observed in both humans and animals, fear can renew when an organism is placed in an environment different than that in which extinction occurred. The contextual modulation of fear involves a tripartite neural circuit consisting of the ventral hippocampus (VH), prelimbic cortex (PL) and basal amygdala (BA). Neurophysiological recordings within the basal amygdala suggest that there are separate populations of neurons active during the renewal or suppression of fear. This suggests that extinction creates unique cell assemblies in the amygdala, and that these assemblies are active in a context-dependent manner. However, it is not clear whether 1) the emergence of these assemblies depends on extinction training, 2) what proportion of neurons within the BA exhibit segregated versus overlapping representations of the CS, and 3) whether this pattern of activity is unique to the amygdala or also occurs in the VH and PL.
Methods: To explore these questions, we used cellular compartment analysis of fluorescent *in situ* hybridization (catFISH). This technique uses the cellular expression profile of the immediate early gene, *Arc*, to visualize neuronal activation patterns to different behavioral experiences. Rats were fear conditioned in one context (context A) and extinguished in another (context B or C; EXT). A separate group of rats were also conditioned but did not undergo extinction (NE). Twenty-four hours after extinction, EXT rats were sequentially tested in the extinction context (SAME) and outside the extinction context (DIFF) across a thirty-minute time period (test order counterbalanced). Non-extinguished rats also received two tests that occurred in the two different test contexts.
Results: As we have previously reported, *Arc* expression reflected the context-dependent modulation of fear: the PL exhibited greater numbers of *Arc*-positive neurons after fear renewal whereas the infralimbic cortex exhibited the converse pattern. Importantly, extinction training increased the proportion of single-labeled neurons in the PL and BA relative to NE controls, suggesting the emergence of distinct cell assemblies in these areas. This increase was greatest in the BA, in which there were nearly 3 times as many single-labeled relative to double-labeled cells. Interestingly, despite the increase in single-labeled neurons in the PL, the majority of cells in the PL remained double-labeled relative to other brain regions. This suggests that despite the effects of extinction, the majority of PL neurons responded to the CS independent of where it was presented. Lastly, there were no differences in *Arc*-positive neurons between the extinction recall and renewal conditions in the VH. Furthermore, the ratio of single- to double-labeled cells in the VH was approximately equal, suggesting a heterogeneous mixture of cells engaged during context-dependent retrieval of fear.
Conclusions: Together with our previous work, these findings suggest that context-related neuronal ensembles in the BA may emerge from convergent neural input during extinction training.
Keywords: context, hippocampus, amygdala, prefrontal cortex
Disclosure: C. Orsini, Nothing to Disclose; C. Yan, Nothing to Disclose; S. Josselyn, Nothing to Disclose; S. Maren, Nothing to Disclose.

M69. GABAA and GABAB Receptor Subunits Display Altered Expression in Cerebella of Subjects with Schizophrenia, Bipolar Disorder, and Major Depression

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Background: Gamma-aminobutyric acid (GABA) acts as the main inhibitory neurotransmitter in the central nervous system. GABA signaling is important for multiple processes including learning and memory and GABAergic dysfunction has been implicated in multiple psychiatric disorders. In the current study we have examined expression of $GABA_A$ and $GABA_B$ receptor subunits in the cerebellum, a brain area that has been implicated in the pathology of schizophrenia, bipolar disorder, and major depression.
Methods: Postmortem, lateral cerebellar samples were derived from subjects with schizophrenia (N = 15), bipolar disorder (N = 15), major depression (N = 15) and matched controls (N = 15). SDS-PAGE and western blotting were used to measure protein levels. We measured protein levels of $GABA_A$ receptor alpha 1 (GABRα1), $GABA_A$ receptor alpha 2 (GABRα2), $GABA_A$ receptor alpha 3 (GABRα3), $GABA_A$ receptor beta 1 (GABRβ1), $GABA_A$ receptor beta 2 (GABRβ2), $GABA_B$ receptor 1 (GABBR1), and $GABA_B$ receptor 2 (GABBR2). All protein measurements for subjects with schizophrenia, bipolar disorder, major depression, and control subjects were normalized against b-actin. Group differences were analyzed statistically using student's t-test. Significant differences were defined as those with a p value < 0.05. Confound effects (i.e., age and PMI) were examined using Pearson's correlation test
Results: We observed significant increases in protein for GABRα1 in cerebella of subjects with schizophrenia ($p < 0.049$), bipolar disorder ($p < 0.046$) and major depression ($p < 0.022$). Similarly GABRα2 was also increased in brains of subjects with schizophrenia ($p < 0.0084$), bipolar disorder ($p < 0.026$), and major depression ($p < 0.0054$). In contrast, GABRα3 was not significantly altered in the three diagnostic groups. GABRβ1 protein was reduced in cerebella of subjects with schizophrenia ($p < 0.0006$) and bipolar disorder ($p < 0.021$) while GABRβ2 was significantly reduced in cerebella of subjects with bipolar disorder ($p < 0.014$).

GABBR1 showed reduced expression in cerebella of subjects with schizophrenia ($p < 0.0007$), bipolar disorder ($p < 0.012$), and major depression ($p < 0.023$). Similarly GABBR2 also showed reduced expression in cerebella of subjects with schizophrenia ($p < 0.0001$), bipolar disorder ($p < 0.0063$), and major depression ($p < 0.0020$).
Conclusions: Our results are the first to demonstrate widespread altered expression of $GABA_A$ and $GABA_B$ subunits in the cerebella of subjects with schizophrenia and mood disorders. It is likely that altered GABA receptor expression contributes to the cognitive and behavioral deficits associated with these disorders. Grant support by NIMH (5R01MH086000-02) is appreciated.
Keywords: GABA, Schizophrenia, Bipolar Disorder, Major Depressive Disorder, Cerebellum
Disclosure: S. Fatemi, Nothing to Disclose; T. Folsom, Nothing to Disclose.

M70. Abnormalities of the Ubiquitin-Proteasome System in the Superior Temporal Gyrus in Schizophrenia

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Background: Schizophrenia is a complex psychiatric disorder, whose underlying patophysiology is still unknown. Diverse post- and co-translational modifications, such as palmitoylation, myristoylation, SUMOylation and ubiquitination, are thought to underlie protein expression and trafficking abnormalities seen in this illness. At the mRNA level, several groups have reported abnormalities of the ubiquitin-proteasome system in different areas of the brain. These findings, in addition to the differences in the expression level of specific proteins consistently found schizophrenia, led us to hypothesize that some protein expression abnormalities seen in this illness are a consequence of abnormal protein degradation driven by the ubiquitin-proteasome system.
Methods: We studied protein expression of the ubiquitin-proteasome system by performing Western blot analysis of the superior temporal gyrus (STG) in paired subjects with schizophrenia or a matched control. We measured overall protein ubiquitination, overall K63 and K48 linked ubiquitination and free ubiquitin. Based on previous peripheral blood mRNA findings, we also studied the expression of E1 activases, E2 conjugases and E3 ligases.
Results: Our results show an overall reduction in protein ubiquitination accompanied by a decrease in free ubiquitin in schizophrenia. Although overall K63 and K48 linked ubiquitination was not modified, individual band analysis yielded differences at specific molecular weights in schizophrenia. We also found a decrease in E1 activases UBA3, UBA6, MOCS3, ATG7 and NAE1 and E3 ligases Nedd4 and USP2 in this group.
Conclusions: This study of the ubiquitin-proteasome pathway showed abnormalities in ubiquitination in the STG in schizophrenia postmortem brains. These findings are likely to be the result of defective ubiquitin activation and subsequent ligation, given the decrease in the expression of E1 activases and E3 ligases. The defects in the ubiquitin-proteasome pathway we report may underlie of abnormal protein expression in schizophrenia.
Keywords: proteins, degradation pathway, patophysiology
Disclosure: M. Rubio, Nothing to Disclose.

M71. ChIP-Seq Analysis Identifies Genome-wide Binding of Histone 4 Acetylated at Lysine 5 (H4K5ac) as a Mediator of the Acute Transcriptional Effects of Methamphetamine

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Background: Methamphetamine (METH) addiction is a chronic relapsing neuropsychiatric disorder. Acute METH administration induces the expression of several genes including immediate early genes (lEGs) and neuropeptides. Although this is a well established phenomenon, the epigenetic bases of these METH-induced changes in gene expression remain to be clarified. Regulation of gene expressionn depends, in part, on epigenetic modifications that include histone acetylation and methylation. Presently, it is possible to measure epigenetic changes on a global level by using chromatin immunoprecipitation (ChIP) followed by large-scale sequencing (Seq).
Methods: In the present study, we used microarray and ChIP-Seq analyses to quantify global gene expression and DNA binding of acetylated histone H4 at lysine 5 (H4K5ac), respectively, after acute and chronic METH administration.
Results: We found that acute METH caused induction of several lEGs and neuropeptides. There were also large-scale changes in H4K5ac-DNA binding around transcription start sites (TSS) of 18 of these genes. Quantitative PCR validated the changes in gene expression. In contrast, in spite of marked changes in H4K5ac binding in the chronic group, only one gene showed parallel increases in expression and H4K5ac-TSS binding. Interestingly, acute METH injection to chronic METH-treated rats caused changes in the expression in a different set of genes and these increases were not consequent to METH-induced increases in H4K5ac binding.
Conclusions: These findings indicate that increased H4K5ac-DNA binding play an important role in mediating the acute effects of METH in METH-naïve but not in METH-pretreated rats. These findings also suggest that histone and/or DNA methylation might be responsible for the chronic effects of METH on gene expression.
Keywords: Methamphetamine, epigenetics, histone acetylation, dorsal striatum, addiction, immediate early genes
Disclosure: J. Cadet, Nothing to Disclose; C. Brannock, Nothing to Disclose; M. McCoy, Nothing to Disclose; S. Jayanthi, Nothing to Disclose; K. Becker, Nothing to Disclose; S. De, Nothing to Disclose; E. Lehrman, Nothing to Disclose.

M72. Heat Shock Protein Hsp90α: A Novel Target for Aripiprazole-induced Neurite Outgrowth

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Background: Aripiprazole is an atypical antipsychotic drug approved for the treatment of psychiatric disorders such as schizophrenia, bipolar disorder, major depressive disorder, and autism. The drug shows partial agonistic activity at dopamine D_2 receptors and 5-hydroxytryptamine 5-HT_{1A} receptors, and antagonistic activity at 5-HT_{2A} receptors. However, the precise mechanistic pathways remain unclear. In this study, we examined the effects of aripiprazole on neurite outgrowth.
Methods: The effects of aripiprazole on NGF-induced neurite outgrowth in PC12 cells were examined. Furthermore, the role of Ca^{2+} signaling via IP_3 receptors and cellular signaling pathways were also examined. Moreover, we performed the proteomic analysis to determine a novel target of aripiprazole.
Results: Aripiprazole significantly potentiated NGF-induced neurite outgrowth in PC12 cells, in a concentration dependent manner. The 5-HT_{1A} receptor antagonist WAY-100635, but not the dopamine D_2 receptor antagonist sulpiride, blocked the effects of aripiprazole. Specific inhibitors of IP_3 receptors and BAPTA-AM, a chelator of intracellular Ca^{2+}, blocked the effects of aripiprazole. Moreover, specific inhibitors of several common signaling pathways (PLC-g, PI3K, mTOR, p38 MAPK, JNK, Akt, Ras, Raf, ERK, MAPK) also blocked the effects of aripiprazole. Using proteomic analysis, we found that aripiprazole significantly increased levels of the heat shock protein Hsp90α in cells. The effects of aripiprazole on NGF-induced neurite outgrowth were significantly attenuated

by treatment with Hsp90α RNAi, but not by the negative control of Hsp90α.
Conclusions: These findings suggest that both 5-HT_{1A} receptor activation and Ca^{2+} signaling via IP_3 receptors, as well as their downstream cellular signaling pathways play a role in the promotion of aripiprazole-induced neurite outgrowth. Furthermore, aripiprazole-induced increases in Hsp90α protein expression may form part of the therapeutic mechanism for this drug.
Keywords: Aripirazole, Neurite outgrowth, Ca2 + signaling, IP3 receptors, Heat shock protein
Disclosure: K. Hashimoto, Nothing to Disclose; T. Ishima, Nothing to Disclose.

M73. Cognitive Impairments Induced by Brief Sleep Deprivation Can be Prevented by Targeting a Single Phosphodiesterase Isoform Selectively in Excitatory Neurons in the Hippocampus

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Background: Millions of people regularly obtain insufficient sleep which results in cognitive impairments. Therefore understanding the cellular and molecular pathways affected by sleep deprivation is of great social and clinical importance. We previously showed that 5 hours of sleep deprivation leads to attenuation of the cAMP pathway in the hippocampus which is reversed by systemic delivery of the non-specific phosphodiesterase 4 (PDE4) inhibitor rolipram. In addition, we found that 5 hours of sleep loss results in the upregulation of the PDE4 isoform PDE4A5 at the protein level. However, it remained to be determined whether PDE4A5 is targeting cAMP-containing complexes important for the consolidation of hippocampus-dependent memories.
Methods: We used a viral approach to express a dominant negative catalytically inactive version of PDE4A5 (PDE4A5DN) selectively in hippocampal excitatory neurons to prevent the degradation of cAMP in PDE4A5 containing signaling complexes. In addition, we conducted experiments in which we expressed a truncated version of PDE4A5DN that lacks the N-terminal domain that is required for anchoring and a compartmentalization of PDE4A5.
Results: We found that expression of the PDE5A5DN prevented the cognitive impairments induced by 5 hours of sleep deprivation without affecting exploratory behavior or causing behavioral abnormalities. Because the compartmentalization of PDE4 isoforms is orchestrated through the anchoring of the isoform-specific N-terminal domains, we next repeated the experiment with a truncated PDE4A5DN that lacked this N-terminal region (PDE4A5DNdelta4). We found that expression of PDE4A5DNdelta4 did not rescue the memory deficit induced by 5 hours of sleep deprivation.
Conclusions: These findings indicate that the reversal of the sleep deprivation-induced memory deficit by the catalytically inactive PDE4A5DN is not due to a global reduction of cAMP hydrolyses. Rather, our findings suggest that the PDE4A5DN needs to be anchored to specific intracellular domains to prevent cAMP degradation at those sites, and to reverse the cognitive deficits observed after brief sleep deprivation. These findings indicate that PDE4A5 may be a potential therapeutic target to prevent the cognitive impairments induced by brief sleep deprivation. Future studies will determine which targets of PDE4A5 are ultimately responsible for the cognitive impairments that coincide with sleep loss.
Keywords: cAMP, PDE, PKA, memory, learning
Disclosure: R. Havekes, Nothing to Disclose; J. Choi, Nothing to Disclose; V. Bruinenberg, Nothing to Disclose; G. Baillie, Nothing to Disclose; K. Krainock, Nothing to Disclose; S. Aton, Nothing to Disclose; P. Meerlo, Nothing to Disclose; M. Houslay, Nothing to Disclose; T. Abel, Nothing to Disclose.

M74. Childhood Adversity is Associated with Fewer Immature and Mature Dentate Gyrus Neurons in Treated and Untreated Depressed Subjects but Not in Non-Psychiatric Controls

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Background: In humans, reported childhood adverse events (CAE), including physical or sexual abuse, parental neglect, maternal rejection, loss of primary caregiver, inter-parental violence and inconsistent discipline, increase the risk for psychopathology in adulthood, frequently major depressive disorder (MDD) and suicidal behavior. History of CAE is also associated with poorer antidepressant response in MDD. Childhood is a critical stage, when environmental stressors can have long-lasting effects on neural circuits involved in emotional control, and susceptibility to psychopathology, and may result in smaller hippocampal volume. The underlying histopathologic changes have not yet been elucidated. MDD is also associated with smaller hippocampus and with apoptosis in the entorhinal cortex, subiculum, dentate gyrus (DG), CA1 and CA4. Animals subjected to maternal deprivation in infancy show increased anxiety and depression-like behaviors in adulthood and reduced adult neurogenesis in the DG. Antidepressants reverse the effects of stress on neurogenesis in rodents and increase neural progenitor cell number (NPCs) in major depression. It is not clear if CAE have an effect on hippocampal neuroplasticity or its response to antidepressant administration in human.
Methods: We sought to assess postmortem the impact of reported CAE (before 15 y) on the number of cells at different stages of maturation in the neuronal lineage in the adult hippocampus of untreated MDD, MDD treated with antidepressants and non-psychiatric controls. Each group included subjects with and without CAE. We quantified by stereology (StereoInvestigator software, MBF Biosciences Inc., Williston, VT): mature granule cells labeled with neuronal nuclear marker (NeuN), immature neurons immunostained with polysialylated cell adhesion molecule (PSA-NCAM), mitotic cells identified by Ki67 and NPCs labeled with nestin. The hippocampus was dissected from 2-cm coronal blocks of the right hemisphere, sectioned at 50 μm, and the area of the DG used as the region of interest for cell counting and volume measures. We assayed sections every 2 mm along the anterior-posterior axis of the DG. Brains had drug toxicology, neuropathological examination and psychological autopsies assessing life adversity history and psychopathology. MANOVA analysis with group and adversity as independent variables tested the main hypotheses.
Results: The number of mature granule neurons (GC) differed between controls, untreated and treated MDDs in the anterior ($F = 6.385$, $df = 2$, $p = .005$) and mid DG ($F = 3.495$, $df = 2$, $p = .042$) such that untreated MDD had lower GC number than the MDD treated and control groups. There was a significant interaction between group and CAE ($F = 3.893$, $df = 2$, $p = .030$), whereby controls with CAE had more GCs than controls without adversity, and MDD with CAE had fewer GCs than those without CAE. Immature neurons differed between groups in the DG body ($F = 4.\ 612$, $df = 2$, $p = .046$), showing an adversity/group interaction ($F = 7.214$, $df = 2$, $p = .016$) whereby CAE was associated with fewer immature neurons in treated MDD but not in controls and untreated MDD. Volume of the anterior ($F = 6.009$, $df = 1$, $p = .027$) and mid DG ($F = 7.016$, $df = 1$, $p = .018$) was smaller in subjects with adversity in treated and untreated MDD ($F = 9.971$, $df = 2$, $p = .002$). NPC and mitotic number showed an effect of group (previously reported) but not of adversity.

Conclusions: In MDD, childhood adversity shows a negative effect on the number of mature and immature DG neurons, as well as on DG volume. On the other hand, numbers of immature and mitotic cells do not appear affected by adversity in this sample. The negative impact of adversity on immature and mature neuron number in treated MDD might be related to differences at the level of the serotonin transporter (5HTT), since MDDs with CAE show lower 5HTT binding potential than those without and carriers of 5HTT *s* allele are more prone to develop MDD, if exposed to adversity, than carriers of the *l* allele. Fewer DG cells might be related to the poorer antidepressant response in MDD exposed to CAE observed in clinical studies, compared with those without CAE. Controls with or without childhood adversity show no difference in GC number or DG volume, and by definition, they have no Axis I or II psychopathology. Therefore, that may be a resilient phenotype, with possible neurobiological responses, to be studied in the future, that are protective regarding the development of MDD following exposure to childhood adversity. Supported by MH83862, MH94888, MH64168, the American Foundation for Suicide Prevention and the Diane Goldberg Foundation.

Keywords: Stress, neurogenesis, hippocampus, stereology, major depression, antidepressant.

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M75. Higher Nestin and GFAP Immunoreactivity in Superior Temporal Cortex in Developing Autism Donors 2-21 Yrs of Age Compared to Age-Matched Controls, Evidence for Sustained Cell and Microvessel Proliferation

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Background: Serotonin, a brain neurotransmitter known to be increased in the plasma of many autism (ASD) patients (Azmitia et al, 2011), functions as trophic factor (Whitaker-Azmitia, 2005). In animal studies, serotonin results in increased cell proliferation (Dizeyi et al., 2005) and neuronal and glial maturation (Azmitia, 2001). Selective serotonin reuptake inhibitors increase cell proliferation and neural progenitor cells (NPCs) in the subgranular zone of the dentate gyrus in human and mice (DG; Boldrini et al. 2012, 2009). Brain samples from autistic donors were previously shown to have increased serotonin fibers (Azmitia et al, 2011), neuron number and macroencephaly (Courchesne, 2012) compared to age matched normally developing individuals (NDI). We hypothesize that the macrocephaly in ASD is produced by serotonin-mediated augmentation of NPCs proliferation leading to more neurons and/or glial cells.

Methods: Brain tissue was obtained from the Brain Bank for Disabilities and Aging in Staten Island, the Autism Tissue Program in Princeton New Jersey, and the NICHD Brain and Tissue Bank for Developmental Disorders at University of Maryland, Baltimore. Superior temporal gyrus (STG) from ASD (n = 6, age 2.8-20.8 yrs.) and NDI (n = 7, age 1.8-20.5 yrs.) donors, was formalin fixed, sectioned at 50 micron, stored in 50% alcohol at 4 C and immunolabeled with anti-nestin mouse monoclonal antibody (1:8000, Chemicon, Temecula, CA) and with anti-GFAP mouse monoclonal antibody (1:9000; Sigma-Aldrich, St Louis, MO). We perform stereology (StereoInvestigator software, MBF Biosciences Inc., Williston, VT) to estimate the density of nestin and GFAP positive cells in a fixed area of layers I-III and in layers IV-V of the STG in each subject, and to measure capillary density in the same areas. Size of NPCs and astrocytes and dendrite density is being analyzed with the nucleator method.

Results: GFAP immunoreactivity in astrocytes in NDI are mainly seen in layer I and in layer VI, and along blood vessels. The GFAP labeled cells are highly branched and in Layer I and VI often contain long processes with numerous spherical varicosities. GFAP immunoreactive cells are increased in layer I-III and in layers IV-V in STG of young ASD subjects (2-8 years) but mainly in Layer I in older donors compared to aged matched NDI. Nestin immunoreactivity is found in NPCs and blood vessels. The labeled NPCs are associated with blood vessels and are foud in layer I and VI. Nestin immunoreactive cells show many processes in ASD and are very rare and smaller in size and processes extension in NDI. Nestin immunoreactive cells are increased in layers I-III and in layers IV-V in STG of young ASD subjects (2-8 years), and capillaries are more abundant and more darkly stained than in NDI. Both nestin and GFAP staining show more cells in ASD of younger age comapred with ASD of older gae.

Conclusions: We have previously shown in postmortem brains an increase in the number of serotonin fibers in cortex and subcortical structures of the telencephalon in ASD (Azmitia et al, 2011). In this report we show an increase in the number of GFAP staining astrocytes, and Nestin labeled NPCs in STC of ASD compared to NDI donors. The labeling is progressive with younger ASD donors showing the most GFAP and Nestin cellular staining. More astrocytes may contribute to larger brain size and weight shown in young ASD subjects. The prominent Nestin labeling of blood vessels occurs in STC of all ASD compared to NDI donors. Angiogenesis is the formation of new capillaries from existing blood vessels, which is essential in brain development and repair. Sustained angiogenesis is believed to be pathological and occurs in tumours and their metastases (Kruger et al, 2012). In postmortem studies of adults who took SSRI there was an increase in Nestin labeling of blood vessels in the hippocampus (Boldrini et al, 2012). The same brains from ASD donors analyzed in this study had previously been shown to have increased serotonin fibers (Azmitia et al, 2011). Thus, it appears likely that the higher Nestin expression in ASD is related to the increase in brain serotonin. The implications of these findings may be relevant to the etiology and treatment of autistic children. Early inhibition of the 5-HT1A receptors may correct this abnormality.

Keywords: Serotonin, astrocytes, angiogenesis, neurogenesis, gliogenesis, cortex, postmortem, macroencephaly, immunocytochemistry, brain

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M76. Stress, PACAP and Epigenetic Control of Adrenergic Function

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Background: Stress induces epinephrine (EPI) release to initiate behavioral and physiological responses that permit an organism to cope with and overcome stress. Stress regulates EPI in part through its biosynthesis by phenylethanolamine N-methyltransferase (PNMT). We have shown that immobilization stress in rats and moderate hypoxic stress (5% oxygen) in adrenal medulla-derived PC12 cells induces the transcriptional activators Egr-1, Sp1 and HIF1α, which, in turn, stimulate PNMT gene expression. Pituitary

adenylate cyclase activating polypeptide (PACAP), which may underlie long-term neural control of EPI, also stimulates the PNMT gene. However, transcriptional activation is orchestrated through Egr-1 and AP-2, accompanied by a rise in HIF1α as well. Otherwise, little is known about mechanisms underlying stress, neural and hormonal control of adrenergic function. The studies to be described were undertaken to further knowledge about potential long-term stress and neural regulatory effects on PNMT and EPI and to examine the possible role of epigenetics in stress- and neural-induced changes in adrenergic function.
Methods: PC12 cells transfected with PNMT promoter-luciferase reporter gene constructs were exposed to PACAP (10 nM), 5% O_2 and 4 mM sodium butyrate (NaBut) alone or in combination for 6 or 24 h and luciferase activity assayed. Alternatively, untransfected cells were similarly treated and total RNA, nuclear protein and cytosolic protein isolated. PNMT and transcription factor mRNA was assessed in total RNA by RT-PCR. Transcription factor and PNMT protein were determined from nuclear or cytosolic protein extracts respectively by western analysis.
Results: All treatments, alone or in combination, markedly activated PNMT promoter-driven luciferase expression and endogenous PNMT mRNA and protein expression. Egr-1, AP-2, Sp1 and HIF1α mRNA and protein showed significant increases as well. The extent of all changes was treatment- and time-dependent with maximum stimulation observed in cells exposed to the combination of PACAP, reduced O_2 and NaBut for 24 h. The latter is consistent with the maximal activation of PNMT and its transcriptional activators by hypoxia occurring at 6 h and that for PACAP and the histone deacetylase inhibitor NaBut occurring at 24 h.
Conclusions: Together, these findings suggest that stress and neural regulation of PNMT and EPI occurs in part through transcriptional activation initiated via induction of the PNMT gene activators, Egr-1, AP-2, Sp1 and HIF1α. Given that the histone deacetylase inhibitor, NaBut, provides equivalent maximal activation to that observed via PACAP or stress, epigenetic effects may be exerted through control of transcription factor expression, likely through HIF1α. We have previously identified HIF1α, an important transcriptional activator in the hypoxic stress cascade, as a master switch regulating transcription of the PNMT gene through control of Egr-1 and Sp1. While the role of the stress hormone EPI has been predominantly associated with mediation of short-term stress responses related to "fight or flight" responses to stress, EPI over expression associates with a variety of stress-related disorders, such as cardiac disease, behavioral illnesses, cancer and immune dysfunction. Thus, sustained over expression of EPI may have adverse effects that lead to maladaption. We hypothesize that when stress-elevation of EPI becomes maladaptive, HIF1α may loose its ability to regulate Egr-1 and Sp1. Findings may provide important insight into the maladaptive processes associated with stress, adrenergic function and disease.
Keywords: epinephrine, PNMT, PACAP, hypoxia, epigenetics
Disclosure: D. Wong, Nothing to Disclose; R. Claycomb, Nothing to Disclose.

M77. Activity-Dependent Phosphorylation of MeCP2 T308 Regulates Interaction with NCoR Co-repressor Complex

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Background: Rett syndrome (RTT) is a neurodevelopment disorder with features of autism that is caused by mutations in *MeCP2*. In addition, less severe mutations in *MeCP2* can lead to a wider spectrum of neuropsychiatric disorders, including autism and psychotic spectrum disorders. MeCP2 is a nuclear protein that binds DNA at methylated cytosines and represses transcription. In neurons, MeCP2 is expressed at high levels, stochiometrically equivalent to core histones and is bound broadly across the genome. The molecular mechanisms of how loss of MeCP2 leads to RTT are not well understood. Neuronal activity triggers the phosphorylation of MeCP2 at S421. While S421A knock-in mice have defects in synapse development and behavior, the mutation had no detected effect on transcription. In addition, the proximal molecular impact of phosphorylation at S421 on MeCP2 is not known. Mass spectrometry studies have revealed many additional sites of phosphorylation in MeCP2; however, no other phosphorylation site has reproducibly been shown to be induced by neuronal activity. We hypothesized that multiple post-translational modifications of MeCP2, bound broadly across the genome, dynamically regulate its activity, modifying transcription and chromatin.
Methods: To identify novel sites of activity-dependent phosphorylation, we used phosphotryptic mapping of MeCP2 derived from ^{32}P-orthophosphate-labeled primary cortical neurons that had been left untreated or membrane-depolarized. To identify the sites of phosphorylation that correspond to the phospho-peptide spots that appeared with membrane-depolarization, we generated phosphotryptic maps of MeCP2, wildtype or with missense mutations at putative sites of phosphorylation, which had been phosphorylated using *in vitro* kinase assays. We generated phospho-site specific antibodies to each site of phosphorylation. We used these antibodies in Western blotting to determine if various stimuli in neuronal cell culture, or *in vivo*, induce the phosphorylation at each site in MeCP2. We used synthetic peptides in pull-down assays and co-immunoprecipitation assays to determine if phosphorylation of T308 altered MeCP2's ability to bind co-factors. We used organotypic hippocampal cultures biolistically transfected with MeCP2 variants to determine the role of phosphorylation of MeCP2 T308 in regulating dendritic arborization.
Results: Using phosphotryptic mapping, we found multiple sites of activity-induced phosphorylation of MeCP2. Phosphorylation of these sites are differentially induced by neuronal activity, brain-derived neurotrophic factor, or agents that elevate the intracellular level of cAMP, suggesting that MeCP2 functions as an epigenetic regulator of gene expression that integrates diverse signals from the environment. By Western blotting with the phospho-site specific antibodies to MeCP2 pT308, we find that the phosphorylation of MeCP2 T308 is induced by neuronal activity upon calcium influx into neurons via L-type calcium channels and NMDA receptors. We find that the common RTT missense mutations at R306, by disrupting the basophilic kinase recognition motif, prevent phosphorylation of MeCP2 T308. Phosphorylation of MeCP2 T308 abrogates an interaction of MeCP2 with NCoR co-repressor complex and impairs the ability of MeCP2 to provide transcription repression. We find that phosphorylation of MeCP2 T308 regulates dendritic arborization, a phenotype altered in RTT.
Conclusions: These findings indicate that neuronal activity induces phosphorylation of MeCP2 at T308 and that phosphorylation at this site disrupts an interaction with NCoR co-repressor complex and regulates MeCP2's ability to mediate transcription repression. The NCoR co-repressor complex contains HDAC3, a histone deacetylase. The regulated interaction between MeCP2 and NCoR may modulate histone acetylation in response to neuronal activity to control transcription in neurons. The phosphorylation of T308 has not been observed previously in mass spectrometry studies from many different laboratories, indicating the utility of phosphotryptic mapping in identifying sites of activity-dependent phosphorylation. The common RTT missense mutations at R306 both disrupt an interaction with NCoR and prevent experience triggered phosphorylation of MeCP2 at T308. The loss of this regulated interaction between MeCP2 and the NCoR co-repressor complex may underlie critical aspects of RTT. Investigation of activity-dependent phosphorylation of MeCP2 may help identify

targets for novel therapeutics for RTT and the broader spectrum of neuropsychiatric disorders caused by mutations in *MeCP2*.
Keywords: Rett syndrome, neuronal activity, autism, activity-dependent phosphorylation, transcription
Disclosure: D. Ebert, Nothing to Disclose; M. Greenberg, **Part 1:** Consulting for Roche in 2011, Spouse consults for Novartis, **Part 4:** Grant from Roche.

M78. Induced Pluripotent Stem Cell (iPSC) Models for Bipolar Disorder

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Background: The generation of cellular models for the study of complex neuropsychiatric disease using stem cell technology offers the opportunity to study affected tissues from a cellular and developmental biology perspective. The goal of this study is to develop and characterize iPSC lines from BP individuals and controls.
Methods: Fibroblast lines were developed from 4 BP and 3 control subjects. Dermal fibroblasts at passage 1-2 were transduced with individual retroviral constructs expressing pluripotency factors: Oct4, Klf4, cMyc, and Sox2. For neural differentiation iPSC were grown in suspension culture in N2 medium ± retinoic acid, as well as a BMP signaling inhibitor and a nodal inhibitor. After 4 days, they were plated, and neural rosettes re-plated on day 21 of differentiation. Neural differentiation was assessed using antibodies to: nestin, bIII tubulin, GFAP, and synaptic densities, followed by appropriate secondary antibodies. Fibroblasts were identified using antibodies to Te-7 or P4HA1.
Results: Twelve stock vials of fibroblasts from each patient were banked and have undergone initial characterization. From them, more than 40 iPSC lines have been derived and characterized. With reprogramming, the pluripotency gene Nanog was induced, while levels of fibroblast-restricted genes e.g., Te-7, were down-regulated. Both control and iPSC derived from BP individuals are capable of widespread neuronal differentiation, forming highly branched networks of neurites. Studies of synaptic characteristics and transmembrane voltage changes using optical mapping are in progress, to be followed by SEM and derivation of additional cell lines.
Conclusions: The derivation of iPSC lines from dermal fibroblasts provides an opportunity to develop models of complex neuropsychiatric illness. We describe the derivation and characterization of iPSC lines from patients with Bipolar Disorder, and demonstrate their ability to undergo widespread neuronal differentiation.
Keywords: bipolar iPSC biological modeling
Disclosure: M. McInnis, **Part 1:** Speaker's Bureau Merck Pharmaceuticals; H. CHEN, Nothing to Disclose; C. DeLong, Nothing to Disclose; S. O'Shea, **Part 1:** Consulting for Genetech.

M79. Knockdown of TrkB in the Rat Nucleus Accumbens Alters BDNF Signaling in the Mesocorticolimbic Circuit and Prevents Effects of Social Defeat Stress on Amphetamine Cross-Sensitization

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Background: Brain-derived neurotrophic factor (BDNF) is involved in neuroplasticity induced by stress and drugs of abuse. BDNF released from the ventral tegmental area (VTA) and the prefrontal cortex (PFC) exerts a cellular effect mediated through TrkB receptors in the nucleus accumbens (NAc). We previously showed that repeated exposure to social defeat stress persistently increases BDNF level in the VTA, as well as BDNF/deltaFosB co-expression in mesocorticolimbic terminal regions. These cellular changes are accompanied by behavioral cross-sensitization to amphetamine. We hypothesized that BDNF-TrkB signaling in the NAc is required for social defeat stress-induced cross-sensitization to amphetamine
Methods: We used adeno-associated virus-mediated gene transfer to deplete the TrkB level in the NAc by overexpression of short hairpin RNA directed against TrkB. AAV-shTrkB or control AAV-GFP constructs were infused bilaterally into the NAc shell of Sprague-Dawley rats. The resident-intruder model was used. Intermittent social defeat stress consisted of four brief confrontations between the experimental intruder rat and an aggressive resident rat over the course of 10 days. Control animals were handled according to the same schedule. Amphetamine (1.0 mg/kg, ip) challenge was performed ten days after the last stress or handling procedure. Western blot analyses of BDNF, TrkB, deltaFosB and phospho-ERK, were performed in the NAc, VTA, and PFC.
Results: Knockdown of TrkB in the NAc shell prevented stress-induced cross-sensitization to amphetamine and reduced phospho-ERK expression in the NAc, thereby confirming the efficiency of TrkB knockdown. Moreover, TrkB knockdown in the NAc prevented social defeat stress-induced elevation of BDNF in the VTA and reduced BDNF level in the PFC. BDNF level in the NAc was significantly lower in AAV-shTrkB animals than in AAV-GFP animals, which is consistent with a reduction of BDNF afferent supply originating from the VTA and the PFC. However, TrkB protein level was significantly higher in the PFC of AAV-shTrkB rats after repeated brief social defeat, which could represent a compensatory change due to reduced BDNF delivery from the VTA. After social defeat stress exposure, deltaFosB expression was significantly increased in the NAc and the PFC of control rats, but remained unchanged in AAV-shTrkB rats.
Conclusions: Reduction of TrkB expression in the NAc prevented social defeat stress-induced cross-sensitization to amphetamine, and altered BDNF signaling throughout mesocorticolimbic circuits. Inhibition of NAc deltaFosB expression by TrkB knockdown implicates deltaFosB in stress-induced cross-sensitization. ERK is a common element for BDNF, dopamine and glutamate intracellular pathways; reduced ERK phosphorylation after TrkB knockdown might be a key factor that prevents cross-talk between various signaling mechanisms that induce cross-sensitization following social defeat stress. Support contributed by: USPHS award DA026451.
Keywords: Brain-derived neurotrophic factor; nucleus accumbens; social stress; sensitization; mesocorticolimbic circuit
Disclosure: E. Nikulina, Nothing to Disclose; J. Wang, Nothing to Disclose; J. Kleiman, Nothing to Disclose; E. Terwilliger, Nothing to Disclose; C. Bass, Nothing to Disclose; R. Hammer, Nothing to Disclose.

M80. N-methyl-D-aspartate Receptor Function and Cocaine-induced Conditioned Place Preference: Implications for Comorbid Schizophrenia and Substance Abuse

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Background: Schizophrenia and substance abuse are highly comorbid disorders. Evidence suggests that the pathophysiology of schizophrenia may increase susceptibility to drug addiction. In fact, abnormalities in glutamatergic signaling are characteristic of both schizophrenia and substance abuse. Specifically, a prominent hypothesis of schizophrenia proposes that the *N*-methyl-D-aspartate receptor (NMDAR) displays decreased activity in patients with schizophrenia compared to healthy controls. In part, this "glutamatergic hypofunction" may be due to decreased

availability of D-serine, an NMDAR co-agonist, as the genes encoding serine racemase (SR; the synthetic enzyme for D-serine), D-amino acid oxidase (DAAO; the degradation enzyme for D-serine), and DAAO activator (G72) are risk genes for schizophrenia. NMDARs also have been implicated in the aberrant regulation of synaptic plasticity evident in substance abuse.
Methods: Our laboratory has developed two transgenic mouse lines to investigate the role of the NMDAR in schizophrenia and substance abuse. The first line is a constitutive knockout of SR. Null mutants (SR−/−) from this line exhibit NMDAR *hypo*function. The second line is a constitutive knockdown of the glial transporter glycine transporter 1 (GlyT1), which removes glycine, another NMDAR co-agonist, from the synapse. Heterozygous mutants (GlyT1+/−) from this line exhibit NMDAR *hyper*function. Both SR−/− and GlyT1+/− mice were tested in a cocaine-induced (20 mg/kg) conditioned place preference (CPP) paradigm. Extinction of CPP, cocaine-induced reinstatement of CPP, and sensitization to the locomotor effects of cocaine were examined concomitantly.
Results: All mice exhibited normal acquisition of cocaine-induced CPP. However, GlyT1+/− mice showed greater locomotor sensitization to cocaine compared to wildtype (WT) littermates and, consistent with our previous work, GlyT1+/− mice exhibited hastened extinction of CPP. In contrast, both GlyT1+/− and WT mice showed robust cocaine-induced reinstatement of CPP. Interestingly, SR−/− mice demonstrated immediate extinction of CPP and were resistant to cocaine-induced reinstatement, even though they exhibited similar locomotor sensitization to cocaine compared to WT littermates.
Conclusions: These results suggest that the glutamatergic system may provide a mechanism for the dissociation of the locomotor activating and rewarding effects of cocaine. As such, NMDAR dysfunction may serve as the neural substrate underlying comorbid schizophrenia and substance abuse. This research was supported by DA015036 and MH51290.
Keywords: schizophrenia, substance abuse, conditioned place preference, NMDA receptors, D-serine
Disclosure: M. Puhl, Nothing to Disclose; A. Bechtholt, Nothing to Disclose; J. Coyle, **Part 1:** Abbott, Jansen Pharmaceutical, Puretech, En Vivo, A patent owned by Massachusetts General Hospital for the use of D-serine as a treatment for serious mental illness could yield royalties for Dr. Coyle.

M81. "Erasing" a Cocaine-cue Memory in Mice: Potential Implications for Relapse to Drug Taking

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Background: One significant obstacle for the treatment of drug addiction is the high incidence of relapse to drug-taking following months, or even years, of abstinence. Exposure to stimuli that were previously associated with prior drug use can awaken powerful memories that may trigger drug craving and provoke a relapse. Therefore, understanding how animals learn and remember the association between a cue and a drug of abuse (such as cocaine) is a crucial step to develop more effective treatment strategies for preventing and treating relapse in humans. Here we examined the neural mechanisms that mediate how cues become associated with the rewarding properties of cocaine to determine if disrupting expression of this cue-reward memory can help prevent relapse.
Methods: CREB (cAMP/Ca^{2+} responsive element binding protein) is a transcription factor that has a well-documented role in neuronal plasticity and long-term memory formation. Previously we found that increasing levels of the transcription factor CREB in a subset of lateral amygdala (LA) neurons in mice enhanced the formation of a fear memory and that selectively ablating these neurons post-training essentially "erased" the fear memory (Han et al., *Science*, 2007, 2009). We took advantage of this approach to investigate whether LA neurons are also critically involved in a cocaine-cue associated memory. To assess cocaine-cue memory, we used the conditioned place preference (CPP) paradigm. In this task, an otherwise neutral environment is paired with cocaine administration. A second neutral environment is paired with saline administration. Drug-free mice are then given the opportunity to spend time in each of these environments. Mice that have learned and remember the association between the particular environment and cocaine spend more time in this drug-paired environment.
Results: To increase CREB function in a subset of LA neurons, we microinjected replication-defective herpes simples virus (HSV) vectors encoding CREB or GFP (control) into the LA of mice. Increasing CREB in a small subset of LA neurons during (but not after) conditioning (pairing cocaine with a neutral environment) enhanced cocaine-CPP memory. To determine if these LA neurons with increased CREB function comprised a crucial component of the "cocaine memory-trace", we used inducible diphtheria toxin receptor (iDTR) transgenic mice to selectively ablate just these neurons after conditiioning. Deletion of LA neurons with increased CREB function (but not a similar proportion of random neurons) blocked expression of a previously acquired cocaine-CPP memory. That is, we were able to disrupt expression of a cocaine CPP by simply ablating a small portion of LA neurons that overexpressed CREB during conditioning. In contrast to extinction training, the disruption of CPP produced by ablating neurons overexpressing CREB was resistant to reinstatement following a low priming dose of cocaine. These findings suggest that a critical component of the cocaine-CPP memory is essentially erased. Next, rather than (irreversibly) ablating these neurons overexpressing CREB, we took advantage of the DREADD (designer receptors exclusively activated by designer drug) system to temporarily inactivate neurons overexpressing CREB. hM4Di is an engineered receptor that is coupled to Gi protein; binding of hM4Di by clozapine-N-oxide (CNO), an otherwise pharmacologically inert compound, promotes neuronal inhibition. We microinjected viral vectors expressing both CREB and hM4Di and found that "silencing" neurons overexpressing CREB before CPP testing similarly inhibited the expression of cocaine CPP memory.
Conclusions: Our results indicate that, similar to a conditioned fear memory, a small population of LA neurons is critically involved in a cocaine-associated memory. Not only do the results of these studies inform us as to the biology underlying the development and expression of cue-cocaine associations, but, in the future, these findings could serve as a foundation for the development of new pharmacotherapies aimed at treating or even preventing drug relapse.
Keywords: mouse model addiction cocaine amygdala relapse
Disclosure: S. Josselyn, Nothing to Disclose; H. Hsiang, Nothing to Disclose; M. van den Oever, Nothing to Disclose; C. Yan, Nothing to Disclose; A. Rashid, Nothing to Disclose; P. Frankland, Nothing to Disclose.

M82. Tricyclic Antidepressant Amitriptyline Indirectly Increases the Proliferation of Adult Dentate Gyrus-derived Neural Precursor Cells through Inducing FGF2 Secretion from Astrocytes

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Background: Antidepressants increase neurogenesis in adult dentate gyrus (DG), which is considered to be involved in the therapeutic action of antidepressants. However, the mechanism underlying it remains unclear. Using the culture system of adult rat DG-derived neural precursor cells (ADP), we have already shown that antidepressants have no direct effects on ADP. Therefore,

antidepressants may increase neurogenesis in adult DG with unknown indirect mechanisms. We have also shown that Amitriptyline (AMI), a common tricyclic antidepressant, increases the secretion of GDNF, BDNF, FGF2 and VEGF from cultured astrocytes. All of GDNF, BDNF, FGF2 and VEGF increase *in vivo* neurogenesis in DG of adult rodents. These suggest that AMI-induced factors in astrocytes may increase neural precursor cells in adult DG. To examine this hypothesis, we examined the effects of GDNF, BDNF, FGF2, VEGF and conditioned medium (CM) from primary cultured astrocytes (PCA) treated with AMI on ADP proliferation.

Methods: ADP were prepared from adult DG of adult SD rat and cultured with Neurobasal-based medium. PCA were prepared from hippocampus of postnatal Wister rat and cultured with DMEM-based medium. CM was prepared by culturing PCA with Neurobasal-based medium. When the effects of CM on ADP proliferation, CM and Neurobasal-based medium were equally mixed. The effects of CM, factors and drugs on ADP proliferation were examined with BrdU immunostaining.

Results: AMI had no direct effect on ADP proliferation, but AMI-treated CM increased ADP proliferation in response to the concentration of AMI treated in PCA. Thus, AMI may increase ADP proliferation not directly but indirectly through inducing BDNF, GDNF, FGF2 and VEGF from PCA. Next, the expression of the receptors of BDNF, GDNF, FGF2 and VEGF in ADP were examined with RT-PCR. The receptors of GDNF, BDNF and FGF2, but not VEGF, were expressed in ADP. Thus, the direct effects of BDNF, GDNF and FGF2 were examined with BrdU immnostaining. FGF2 significantly increased ADP proliferation, but not BDNF and GDNF. To confirm that AMI-induced FGF2 mediates the increasing effects of AMI-treated CM on ADP proliferation, the effects of SU5402, a specific inhibitor of FGF receptors and anti-FGF2 antibody on ADP proliferation were examined. Both SU5402 and anti-FGF2 antibody significantly canceled the increasing effects of AMI-treated CM on ADP proliferation.

Conclusions: Our present study has shown that AMI increases ADP proliferation not directly but through inducing FGF2 secretion from PCA. FGF2 in brain is mainly derived from astrocyte. Astrocyte is a key component of the neurogenic niches in adult DG. Therefore, antidepressants may increase *in vivo* neurogenesis in adult DG through inducing FGF2 secretion from astrocyte.

Keywords: antidepressants, adult neurogenesis, neural precursor cell, proliferation, astrocyte, FGF2

Disclosure: S. Boku, Nothing to Disclose; K. Hisaoka-Nakashima, Nothing to Disclose; S. Nakagawa, Nothing to Disclose; A. Kato, Nothing to Disclose; N. Kajitani, Nothing to Disclose; T. Inoue, Nothing to Disclose; M. Takebayashi, Nothing to Disclose.

M83. Redox Dysregulation in Fast-Spiking Interneurons Disrupts Cortical Stability

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Background: Fast-spiking interneuron deficiency is frequently reported in schizophrenic brains. Recently, redox dysregulation has been implicated in these GABA neuron defects in animal studies. However, the physiological consequences of these deficits have not been well characterized. Proper maturation of fast-spiking interneurons is normally required for defining critical periods of experience-dependent cortical plasticity to establish adult behaviors. Accordingly, the disruption of fast-spiking interneurons has been shown to impact the timing and quality of critical period development in the visual cortex. Considering the link between redox dysregulation, parvalbumin (PV)-interneuron deficits, and its impact on critical period timecourse, we hypothesized that a redox dysregulation *within* developing PV-cells alone (as opposed to more global manipulations tested previously) might suffice to cause damaging consequences on cortical development. Here, we genetically induced redox dysregulation specifically within PV-cells *in vivo* and examined the impact on critical period plasticity using the visual system as a model.

Methods: We conditionally deleted the *Gclc* (glutamate cysteine ligase catalytic subunit) gene, which is an ubiquitously expressed rate limiting enzyme producing the major antioxidant, glutathione. Glutathione activity is reduced in patients with schizophrenia, and the GAG-repeat of the *Gclc* promotor is associated with schizophrenia (Gysin et al., 2007). Crossing mice with the *Gclc* gene flanked by loxP sites to animals expressing Cre recombinase specifically in PV-cells produced a progressive *Gclc* deletion in PV-cells from 34% at postnatal day P20 to 70% in adulthood ($>$P50), as evaluated by double *in situ* hybridization for Gclc and Cre mRNA. As a result of *Gclc* deletion, a cell-autonomous enhancement of oxidative stress was observed by 8-oxodg staining in the majority (69%) of PV-cells by post-adolescence. Using this mouse line, immunohistochemical and *in vivo* electrophysiological analysis were performed.

Results: PV-cells are known to be increasingly enwrapped by perineuronal nets of extracellular matrix with the progression of the critical period (P21-35). We observed a significant reduction of perineuronal nets as revealed by WFA staining in the adult visual cortex of PV-Gclc KO mice. Importantly, perineuronal nets contribute to the closure of critical period plasticity. We then assessed experience-dependent visual cortical plasticity in PV-Gclc KO mice after P50 when the majority of PV cells lacked the *Gclc* gene. Extracellular single-unit recording from the binocular zone of visual cortex revealed significant ocular dominance plasticity following short-term (4 day) monocular deprivation in the PV-Gclc KO mice despite being past the peak of their critical period (Contralateral Bias Index(CBI) $= 0.57$ vs 0.68 in PV-Gclc WT, $p < 0.01$). Consistent with only a small amount of *Gclc* deletion in the critical period *per se*, there was no difference in plasticity level at those younger ages. In contrast, CamK2-Gclc KO mice, carrying a severe redox dysregulation restricted to pyramidal cells, showed no adult plasticity as in wild-type mice (CBI $= 0.69$ vs 0.67 CamK2-Gclc WT, $p > 0.4$).

Conclusions: Our results reveal a prolonged period of brain plasticity – or failure to stabilize cortical circuits – in adult animals under conditions of PV-cell specific redox dysregulation *in vivo*. This reflects a redox state-dependent failure to fully enwrap PV-cells with perineuronal nets, which normally balance plasticity and stability of cortical circuits across development. Divergent genetic (e.g. *Gclc, Gclm, PGC-1a, Disc1*) and environmental (e.g. infection, stress, social isolation, ketamine) risk factors have been shown to unbalance oxidative stress and antioxidant defense systems. A recent study confirms a deficit in perineuronal nets in the human schizophrenic brain (Pantazopoulos et al., 2010). Our findings suggest that redox regulators may collectively impact critical period brain development and plasticity. Taken together, failure to curtail circuit rewiring post-adolescence through redox dysregulation may be a novel intermediate phenotype as well as contributing factor to the pathophysiology of schizophrenia.

Keywords: cortical plasticity, criticial period, oxidative stress, parvalbumin, schizophrenia

Disclosure: H. Morishita, Nothing to Disclose; H. Cabungcal, Nothing to Disclose; Y. Chen, Nothing to Disclose; K. Do, Nothing to Disclose; T. Hensch, Nothing to Disclose.

M84. A New Role of Dopamine D2 Receptors in the Regulation of Synaptic Connections

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Background: Schizophrenia is a serious mental disorder affecting millions of people worldwide. Cognitive impairment is a core symptom of schizophrenia and is thought to stem from defective neuronal connectivity. Intriguingly, several groups have reported that the density of dendritic spines in the prefrontal cortex and the hippocampus is reduced in schizophrenic subjects. The dendritic spine deficit is expected to alter synaptic connections between neurons and hence contribute to cognitive impairment in schizophrenia. However, the mechanisms underlying the spine pathology of schizophrenia remains unclear. The dopamine system has long been implicated in schizophrenia. Dopamine acts through D1-like (D1 and D5 type) and D2-like (D2, D3 and D4 type) dopamine receptors. By regulating multiple intracellular signaling pathways, D2R plays a modulatory role in synaptic transmission.
Methods: We investigated the role of D2R in spine morphogenensis and its implication for the pathophysiology and treatment of schizophrenia. Wild-type and sandy mice which has deficient expresseion of the schizophrenia risk gene dysbindin were injected with D2R agonist or antagonist. The effects of D2R activation and inhibition on dendritic spines were examined in hippocampal slices by diolistic labeling.
Results: By activating D2R with pharmacological and genetic approaches, we identified a surprising age-dependent function of D2R in controlling dendritic spine morphogenesis. Interestingly, D2R regulates spines only during a critical period during development, but not in adulthood. In addition, we find in sandy mice with deficient expression of the schizophrenia risk gene dysbindin that D2R hyperactivity is responsible for the spine defect, which can be alleviated by treatment with antipsychotics before adulthood.
Conclusions: These findings identify a novel function of D2R in the structural development of neurons, provide evidence that abnormal D2R activity contributes to disturbances of neural circuits in schizophrenia, and propose a critical temporal window for interventions to prevent the spine pathology of schizophrenia.
Keywords: schizophrenia, D2 receptor, dysbindin, dendritic spine, development
Disclosure: Z. Li, Nothing to Disclose.

M85. Altered Frontal Cortex Insulin Receptor Mediated Signaling and Associated Epigenetic Modifications in Alzheimer's Patients but Not during Aging

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Background: An imbalance in the membrane arachidonic acid (AA) and docosahexaenoic acid (DHA) ratio is assocaited with altered insulin receptor function. Recently, we reported an upregulated AA metabolism and DHA loss in Alzheimer's patients compared to aged matched controls. However, aged control brains did not show similar changes compared to the middled aged brains. An altered insulin receptor function has been implicated in the accumulation of plaques and tangles in AD patients. Insulin/anti-diabetes drugs have some beneficial effects in correcting the cognitive impairments in AD patients. However, the underlying molecular brain insulin receptor mediated signaling during aging and in AD is not agreed upon.
Methods: We hypothesized that unlike in aging brain, AD brain will show dysregulated insulin receptor mediated signaling. In addition, we hypothesized that changes occuring in AD may be influenced by epigenetic modifications. To test these hypotheses, we measured protein levels of insulin receptor, phosphoinositide-3 kinase (PI3K), protein kinase B (PKCB), phospho-PKCB, glycogen synthase 3 kinase beta (GSK3β) in the frontal cortex (Bradmman area 9) of middle aged (43 ± 3 SE years; $n = 10$), aged (70.20 ± 3 SE years; $n = 10$) and aged matched AD patients (70.60 ± 3 SE years; $n = 10$). Further we measured mRNA and promoter methylation for insulin receptor and PI3K in all the three groups.
Results: Compared with aged matched frontal cortex, AD patients showed reduced protein levels of PI3K and phopho-PKCB. These changes are associated with reduced mRNA levels of Insulin receptor, PI3K and hypermethylation of insulin receptor gene. Aged group showed a signficant increase in mRNA levels of insulin receptor and PI3K and without significant change in their protein levels or promoter methylation compared to the middle aged groups.
Conclusions: Dysregulated insulin receptor mediated signaling in AD may promote an accumulation of phsohorylated amyloid and neurofilament proteins in the AD patients. However, these changes may not occur in the aged brain compared to the middle aged group. The lack of significant change in the protein levels of insulin receptor and GSK3β in AD frontal cortex was due to progressive relative change in their protein levels in the aged frontal cortex compared to the middle aged group. An altered in AA and DHA ratio and epigenetic mofificcations in insulin receptor may be contributing to the disturbed brain insulin mediated signaling and synaptic plasticity in AD and to recognize the new targets for drug development.
Keywords: Alzheimer's disease, aging, insulin receptor, epigenetics phosphoinoside 3 kinase, GSK3beta.
Disclosure: J. Rao, Nothing to Disclose; A. Jamil, Nothing to Disclose; S. Rapoport, Nothing to Disclose.

M86. Striatal Adenosine Signaling Regulates EAAT2 and Astrocytic AQP4 Expression and Alcohol Drinking in Mice

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Background: Adenosine signaling is implicated in several neuropsychiatric disorders including alcoholism. Among its diverse functions in the brain, adenosine regulates glutamate release and plays an essential role in ethanol sensitivity and preference. However, the molecular mechanisms underlying adenosine-mediated glutamate signaling in neuroglial interaction remain elusive. We have previously shown that mice lacking the ethanol-sensitive adenosine transporter, type 1 equilibrative nucleoside transporter (ENT1), drink more ethanol compared to wild-type mice and have elevated striatal glutamate levels. In addition, ENT1 inhibition or knockdown reduces glutamate transporter expression in cultured astrocytes.
Methods: First, we examined whether the deletion of ENT1 reduces striatal excitatory amino acid transporter 2 (EAAT2) or EAAT1 using qRT-PCR and Western blot analysis. Since ENT1 is expressed in neurons and astrocytes, we used an astrocytic cell line, C8-D1A to confirm that the mRNA and protein expression changes in ENT1 null mice were due specifically to the absence of ENT1 in astrocytes as a result of ENT1 siRNA knockdown or inhibition. Because the astrocyte-specific water channel, aquaporin 4 (AQP4) colocalizes with EAAT2, we investigated whether this colocalization was altered in ENT1 null mice. Finally, we investigated whether ceftriaxone-induced upregulation of EAAT2 and AQP4 expression in the striatum could also alleviate the excessive alcohol drinking phenotype in ENT1 null mice.
Results: Inhibition or deletion of ENT1 reduced the expression of EAAT2 and AQP4. Ceftriaxone, an antibiotic compound known to increase EAAT2 expression and function, elevated not only

EAAT2, but also AQP4 expression in the striatum. Furthermore, ceftriaxone reduced ethanol drinking.
Conclusions: Our present study demonstrates a novel mechanism implicating the primary adenosine transporter, ENT1, in the regulation of astrocyte-specific gene expression. Astrocytes play an essential role in regulating neurotransmitter levels in the synaptic cleft. The removal of glutamate *via* astrocytic EAAT2 is especially critical to neuronal activity and viability. These observations suggest that adenosine homeostasis is critical for astrocytic cellular function. Overall, our findings indicate that adenosine signaling regulates EAAT2 and astrocytic AQP4 expressions, which control ethanol drinking in mice. These data also have implications, not only for alcohol use disorders, but also for other psychiatric disorders.
Keywords: ENT1, Astrocytes, Aquaporin 4, EAAT2, Alcoholism, Ceftriaxone
Disclosure: M. Lee, Nothing to Disclose; C. Ruby, Nothing to Disclose; D. Hinton, Nothing to Disclose; S. Choi, Nothing to Disclose; C. Adams, Nothing to Disclose; N. Kang, Nothing to Disclose; D. Choi, Nothing to Disclose.

M87. Adolescent Social Isolation Impairs Decision-making and Elevates Deep-layer Prefrontal Cortical Dendritic Spine Density in Adulthood

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Background: Considerable evidence indicates that adolescence is a period of vulnerability for the development of multiple psychiatric disorders, which can be commonly characterized by a failure to engage in goal-directed decision-making. Adolescence is also a period of marked structural maturation and refinement of the prefrontal cortex. While contemporary models argue that adolescent psychiatric vulnerabilities emerge as a consequence of the impact of pathological stimuli on adolescent prefrontal cortical development, empirical support remains limited.
Methods: We developed an ethologically-based animal model of adolescent adversity in which female mice are isolated from postnatal days 31-60, and then re-housed in large social groups in adulthood.
Results: Adult mice with a history of social isolation develop stimulus-response habits at the expense of engaging in goal-directed response strategies. Our initial evidence also indicates that adolescent isolation fundamentally redirects the developmental trajectory of dendritic spine maturation in deep-layer prefrontal cortex. Adult-emergent behavioral vulnerabilities can be reversed by application of a Rho kinase inhibitor during adolescence.
Conclusions: Given that Rho kinase inhibition facilitates cytoskeletal reorganization, our findings provide novel direct evidence that structural plasticity during adolescence determines long-term behavioral outcomes.
Keywords: adolescence, habit, prefrontal, orbitofrontal, stress
Disclosure: E. Hinton, Nothing to Disclose; S. Gourley, Nothing to Disclose.

M88. Cell Adhesion Pathway is Implicated in Lithium Treatment for Adolescent Mania via DNA Methylation Alteration

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Background: Adolescent Bipolar Disorder (BD) is characterized by severe mood swings and neurocognitive impairment and affects approximately 1% of the population. Lithium has been a first-line treatment for BD for decades. Two major hypotheses of lithium's mechanism of action include the models of phosphatidylinositol (PI) metabolism and *GSK-3*-mediated signal transduction. However, additional mechanisms are still being investigated as evidenced by *in vitro* and *in vivo* studies which have demonstrated that lithium may also involve neurotransmitter and hormone signaling, ion transport, circadian regulation, apoptosis inhibition and neurotrophic cascades. Evidence for these putative molecular changes is largely derived from studies of animal models or cultured cells. Human studies have suggested candidate genes. However, two genome-wide association studies (GWAS) of lithium response did not detect any significant genome-wide signal. Evidence for the role of DNA methylation in lithium response was initially identified in candidate gene studies where a significant reduction in methylation the promoter region of the brain-derived neurotrophic factor (*BDNF)* gene was observed. This study aims to discover epigenetic biomarkers of lithium treatment outcome in adolescent BD.
Methods: Caucasian adolescent BD with mania ($n = 22$) and healthy controls (HC; $n = 24$) matched on age, sex, IQ, and Tanner stage were included in this study. All subjects were characterized using clinical measures along with blood extracted before and after 8 weeks. Patients were treated with lithium targeting 0.6-1.2 mEq/L serum concentrations to assess adequacy of treatment after five days of dosing. Clinical outcome measures included Young Mania Rating Scale (YMRS), Child Depression Rating Scale (CDRS-R), and Brief Psychiatric Rating Scale (BPRS). We extracted DNA from whole blood. The Illumina's Infinium HumanMethylation450 BeadChip was used to profile DNA methylation of 485K CpG sites. After obtaining the DNA methylation β value (percent methylation) data, we performed data quality control and removed experimental batch effects using ComBat program. Paired-T test and time course analysis of EDGE (Storey et al. 2005) were used to identify differentially methylated CpG sites comparing case group to control group. Further, we focused on the CpG sites with significant methylation alterations in just the patients. In patients, we identified correlations between methylation changes and changed scores on clinical ratings.
Results: (1). We detected 50,295 CpG sites showing altered methylation ($p \leq 0.05$) in treated patients only. 33,038 sites showed nominally significant change in HC only. Relatively small amount of CpG sites (2790) changed in both patient and control groups. Time course analysis identified one CpG site near *CADM3* (a synaptic cell adhesion molecule) reaching FDR $q \leq 0.05$ significance. (2). In Adolescent BD, 7,138 nominally significant associations ($p \leq 0.05$) were detected between the changes of methylation with changes of YMRS, BPRS and CDRS after treatment. These associations did not survive correction for multiple comparisons. One CpG site, near *DOK6* (gene involved in signaling pathway that regulates neuronal growth), showed altered methylation that correlated with all three clinical measures. (3). The cell adhesion pathway is significantly enriched in pathway analysis of genes with CpG methylation alterations correlated with clinical measure changes (enrichment probability FDR $q < 0.05$).
Conclusions: Lithium induced DNA methylation changes of CpG sites. A CpG site near CADM3 showed significant methylation change in lithium-treated patients comparing to controls. By examining correlations of methylation changes with clinical measure changes in patients, and conducting pathway analyses, we identified that the cell adhesion system may be involved in the mechanism of action of lithium in this cohort. Cell adhesion molecules play a significant role in neuronal growth, synapse formation, plasticity, and neurotransmission. In a pathway analysis of genome-wide genetic association studies, Corvin AP (2010) proposed that cell adhesion molecule pathway is enriched in both BD and schizophrenia. Lithium may rescue neuronal function through its impact on DNA methylation of multiple genes in cell adhesion pathway.
Keywords: DNA methylation, lithium, epigenome, YMRS, BPRS, CDRS, cell adhesion

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M89. Lipid Raft Sequestration of the G Protein, Gsα: A Protein-based Platelet Biomarker for Major Depressive Disorder

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Background: Lipid rafts are specialized membrane domains rich in cholesterol and intimately associated with cytoskeletal components. G protein signaling is influenced by these domains, but, depending upon the receptor, G protein, and effector enzyme, they either facilitate or attenuate signaling. We have demonstrated that, for Gs and Gs-coupled receptors (b-adrenergic, VPAC and 5HT-4, -6, -7), lipid rafts attenuate signaling by separating Gsα from adenylyl cyclase. Several lines of investigations from different laboratories suggest a post-synaptic effect of chronic antidepressants and a possible postsynaptic target for these drugs. Data from rats, cultured neural and glial cells, all suggest that the localization of the G protein, Gsα, in lipid rafts is modified by chronic treatment with a number of antidepressant compounds (SSRI, MAOI and tricyclic). Antidepressants facilitate translocation of Gsα from lipid rafts while post mortem studies show increased Gsα in raft fractions from several brain regions of depressed suicide cases relative to controls. In this study, we sought to determine whether raft fractions prepared from platelets of depressed subjects showed enrichment of Gsαin lipid raft fractions.
Methods: Blood from volunteers ($n = 9$) or newly diagnosed MDD subjects ($n = 15$) was collected at the Marche Regional Psychiatric Clinic (Ancona Italy), separated into component fractions (Platelets, RBC and WBC), coded and shipped to Chicago for assay. Platelet Gsα was extracted, sequentially with Triton X-100 (non-raft fraction) and Triton X 114 (raft fraction) and Gsα was identified and quantified by immunoblotting.
Results: Gsα in the lipid raft fraction was significantly ($p < .001$) greater in platelets prepared from depressed subjects than those from normal controls.
Conclusions: This suggests the possible development of a simple blood test to indicate the presence of depression. Furthermore, as chronic antidepressants have been shown to translocate Gsα from lipid rafts in cultured cells, it will be interesting to follow Gsα sequestration in depressed subjects as they receive and respond (or fail to respond) to treatment. It is hypothesized that Gsα will translocate from lipid rafts within one week in showing a positive response to antidepressant treatment at 8 weeks.
Keywords: G proteins, Lipid Rafts, depression, GPCR,
Disclosure: J. Sprouse, **Part 1:** Lundbeck USA, Pax Neuroscience, **Part 2:** Lundbeck USA, **Part 3:** Lundbeck USA; A. Jackson, Nothing to Disclose; R. Donati, **Part 1:** Pax Neuroscience; L. Tonello, Nothing to Disclose; M. Cocchi, Nothing to Disclose; M. Rasenick, **Part 1:** Eli Lilly, Lundbeck, Pax Neuroscience, Sepracor, Quintiles, **Part 4:** Eli Lilly.

M90. A Trial of Prazosin for Combat Trauma PTSD with Nightmares in Active Duty Soldiers Returned from Iraq and Afghanistan

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Background: The authors conducted a 15-week randomized, parallel, double-blind, placebo-controlled trial of the alpha-1 adrenoreceptor antagonist prazosin for posttraumatic stress disorder (PTSD) nightmares, sleep quality, global function and total PTSD symptoms in active duty soldiers following combat deployments to Iraq and Afghanistan.
Methods: Sixty-seven soldiers at Joint Base Lewis McChord, Washington, with combat operations PTSD and frequent trauma nightmares were randomized to prazosin or placebo for 15 weeks. Drug was titrated over 6 weeks by therapeutic response and adverse effects to a possible maximum dose of 5 mg midmorning and 20 mg bedtime. Primary outcome measures were the Clinician Administered PTSD Scale (CAPS) B2 Recurrent Distressing Dreams ("nightmare") item, the Pittsburgh Sleep Quality Index, and the Clinical Global Impression of Change anchored to functioning. Treatment effect on overall PTSD was assessed with the 17-item total CAPS score. Maintenance psychotropic medications and/or supportive psychotherapy were held constant during the trial
Results: Prazosin subjects improved significantly more than placebo subjects in trauma nightmares, sleep quality, global function and total 17-item CAPS score. CAPS B2 nightmare scores decrease from baseline to end point was 3.1 ± 2.4 (mean ± SD) in the prazosin group vs. 0.9 ± 1.7 in the placebo group (ANCOVA $p < 0.001$, 95%, CI for difference in change from baseline [0.9, 3.3]). PSQI decrease from baseline to endpoint was 5.5 ± 3.8 in the prazosin group vs. 2.3 ± 4.5 in the placebo group (ANCOVA $p = 0.009$, 95% CI [0.9, 5.5]. At endpoint, 67% of prazosin subjects were CGIC responders ("markedly" or "moderately" improved) compared to 21% of placebo subjects (logistic regression $p < 0.001$). Change in total CAPS from baseline to endpoint was 23.6 ± 23.8 in the prazosin group vs. 10.7 ± 20.0 in the placebo group (ANCOVA $p = 0.03$, 95% CI [1.4, 25.8]). Prazosin was well tolerated and blood pressure changes over time did not differ between groups.
Conclusions: Prazosin is effective and well tolerated for combat operations PTSD with trauma nightmares in active duty soldiers.
Keywords: PTSD, nightmares, soldiers, Iraq, Afghanistan, prazosin
Disclosure: M. Raskind, Nothing to Disclose; K. Peterson, Nothing to Disclose; T. Williams, Nothing to Disclose; E. Peskind, Nothing to Disclose.

M91. Reduced Mitochondrial Energy Production in Major Depressive Disorder: Associations with the Serotonin Transporter and Glutamine Synthetase Genes

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Background: Proton magnetic resonance spectroscopy (^{1}H-MRS) studies have demonstrated altered concentration of aminoacid neurotransmitters in the occipital brain of patients with major depressive disorder (MDD). However, the functional implications of this alteration in total glutamate and GABA levels are not well understood. To elucidate the underlying neuronal mechanisms, we employed ^{13}C magnetic resonance spectroscopy (^{13}C-MRS) to investigate neurotransmitter fluxes and mitochondrial neuroenergetics in MDD subjects.
Methods: 21 medication-free patients with MDD and 14 age- and gender-matched healthy controls had ^{1}H-MRS and ^{13}C-MRS scans with viable data. A subset of the subjects was genotyped for the serotonin transporter (5-HTTLPR) and glutamine synthetase (GLUL) genes. ^{1}H-MRS measured total glutamate and GABA concentration in a single voxel placed in the occipital cortex. [1-^{13}C]-glucose was infused intravenously during ^{13}C-MRS acquisition, which provided *in vivo* measures of neuronal and astrocytic tricaboxilic acid cycle (V_{TCAn} and V_{TCAa}) for mitochondrial energy

production, GABA synthesis (V_{GAD}), and glutamate-glutamine cycle (V_{cycle}), which is a measure of glutamate release and uptake.
Results: Patients with MDD had a 26% reduction in mitochondrial energy production of glutamatergic neurons [Mean ± *SEM*; MDD $V_{TCAn} = 0.36 \pm 0.03$ mM, Healthy $V_{TCA\ n} = 0.49 \pm 0.05$ mM, $t = 2.30$, $n = 35$, $p = 0.028$]. GABA and glutamate concentrations, V_{cycle}, and V_{GAD} did not differ between groups ($p > 0.1$). Among the MDD subjects, carriers of the short allele of 5-HTTLPR (SS or SL) have reduced neuronal V_{TCA} compared to those homozygous for the long allele (LL) [$t = 2.86$, n = 12, $p = 0.017$]. In addition, we found a significant association [$p < 0.009$] between astrocytic V_{TCA} and 3 GLUL SNPs (rs12136955; rs12735664; rs4652705).
Conclusions: The reduction of glutamatergic neuronal energy production (V_{TCAn}) in the occipital brain of depressed subjects raises two possibilities: (1) reduced activity of glutamatergic neurons in this brain region or (2) impaired mitochondrial function. Although we did not detect a difference in activity (as measured through V_{cycle}), this may be due to the V_{cycle} measurement with 1-^{13}C glucose being less precise than the V_{TCAn} measurement. However with the recent demonstration that combined use of ^{13}C glucose and ^{13}C acetate enhances the precision of measuring V_{cycle} several fold, it would be possible to distinguish these possibilities in future studies, as well as further explore the impact of MDD on the astrocytic TCA cycle. Finally, the serotonin transporter and glutamine synthetase genes were associated with mitochondrial energy production in glutamatergic neurons and astrocytes, respectively. Further exploration, in future studies, of these intriguing preliminary findings may provide insight in the mechanisms through which these genes affect cerebral function and psychopathology.
Keywords: Major Depressive Disorder, 13C Magnetic Resonance Spectroscopy, Neuroenergetics, Mitochondrial Function.
Disclosure: C. Abdallah, Nothing to Disclose; G. Mason, Nothing to Disclose; H. De Feyter, Nothing to Disclose; M. Fasula, Nothing to Disclose; B. Kelmendi, Nothing to Disclose; A. Simen, **Part 1:** Work for Merck, **Part 2:** Work for Merck, **Part 3:** Work for Merck, **Part 4:** Work for Merck; L. Jiang, Nothing to Disclose; J. Krystal, Nothing to Disclose; D. Rothman, Nothing to Disclose; G. Sanacora, **Part 1:** G.S. has received consulting fees from Abbott, AstraZeneca, Avanier Pharmaceuticals, Bristol-Myers Squibb, Evotec, Eli Lilly & Co., Hoffman La-Roche, Johnson & Johnson, Novartis and Novum Pharmaceuticals over the past 24 months. He has also received additional grant support from AstraZeneca, Bristol-Myers Squibb, Hoffman La-Roche, Merck & Co. and Johnson and Johnson over the past 24 months. In addition, he is a co-inventor on a filed patent application by Yale University (PCTWO06108055A1).

M92. Social and Non-social Cognition in Bipolar Disorder and Schizophrenia: Relative Levels of Impairment

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Background: There is a long-standing debate about whether bipolar disorder and schizophrenia should be considered separate diseases or different manifestations of the same pathophysiological process. Cognition is a strong determinant of functioning in both disorders; however, the magnitude of impairment for different domains of cognition across disorders is not well understood. This study aimed to determine the relative extent of impairment in social and non-social cognitive domains in patients with bipolar disorder compared to patients with schizophrenia and healthy controls.
Methods: Sixty-eight clinically stable outpatients with bipolar disorder, 38 clinically stable outpatients with schizophrenia, and 36 healthy controls completed a range of social cognitive tasks (i.e. facial affect perception, emotional regulation, empathic accuracy, mental state attribution, self-referential memory) and non-social cognitive tasks (i.e. speed of processing, attention/vigilance, working memory, verbal memory, visual memory, and reasoning/problem solving).
Results: First, for each social cognitive task, patients with bipolar disorder did not differ significantly from controls, and both groups performed better than schizophrenia patients. Within the bipolar group, social cognitive performance was not related to clinical features and medication status (i.e. bipolar I versus II; bipolar I with a history of psychosis versus bipolar I without such a history; bipolar patients taking antipsychotic medications versus those without taking antipsychotics). Second, when evaluating patterns of performance across the tasks (i.e. profiles) within the domains of social and non-social cognition, patients with bipolar disorder showed performance profiles that were similar to controls on both social and non-social cognitive domains whereas both groups differed from schizophrenia patients for both domains. Third, when comparing the relative impairments across social and non-social cognitive domains using a composite score, bipolar patients showed intermediate performance between schizophrenia patients and controls across domains. Further, we found a significant domain by group interaction showing that bipolar patients performed significantly better on social versus non-social cognitive domains, whereas schizophrenia patients showed the opposite pattern.
Conclusions: Patients with bipolar disorder showed less impairment on social cognitive than non-social cognitive performance, whereas schizophrenia patients showed more impairment on social versus non-social cognitive performance. These results suggest that these two cognitive domains play different roles in bipolar disorder versus schizophrenia.
Keywords: social cognition, non-social cognition, bipolar disorder, schizophrenia
Disclosure: J. Lee, Nothing to Disclose; L. Altshuler, **Part 1:** Dr. Altshuler has received advisory board honoraria from Sepracor and consulting fees from Eli Lilly; D. Glahn, Nothing to Disclose; D. Miklowitz, **Part 1:** Dr. Miklowitz has received speaker's fee from GlaxoSmithKline pharmaceuticals and book royalties from Guilford Press and John Wiley and Sons, Inc.; K. Ochsner, Nothing to Disclose; M. Green, **Part 1:** Dr. Green reports having received consulting fees from Abbott Laboratories, Amgen, Cypress, Lundbeck, Shire, and Teva, and speaking fees from Otsuka and Sunovion.

M93. Reducing GABA-A Alpha 5 Receptor-mediated Inhibition Restores Synaptic Plasticity and Neuromorphological Deficits in a Mouse Model of Down Syndrome

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Background: Down syndrome (DS) is the most common genetic cause of intellectual disability. There is currently no pharmacological therapeutic option available for the treatment of cognitive deficits in people with DS. The genetic dependence of the cognitive phenotype in DS has been recapitulated in mouse models of the condition of which the Ts65Dn (TS) mouse is the most widely used. This murine model shows several fundamental features of DS including learning and memory deficits with alterations in both hippocampal morphology and adult neurogenesis. We have shown that RO4938581, a selective GABA-A alpha 5 negative allosteric modulator (also called inverse agonist), has cognition-enhancing properties in the TS mouse. The aim of the current study was to

evaluate whether chronic treatment with RO4938581 affects synaptic plasticity and neuromorphological deficits of the TS mouse model of DS.
Methods: RO4938581 was administered orally for 6 weeks to 3-4 months old TS and euploid littermates (CO) mice. To assess the modulatory effect induced by chronic treatment of RO4938581 on a model of synaptic transmission, long-term potentiation (LTP) was performed in mouse brain slices. Since impairment in hippocampal cell proliferation and neurogenesis is a major pathological hallmark in DS and TS mice, we first assessed if cell proliferation in the DG was affected by chronic treatment of RO4938581 in TS and CO mice. We used Ki67 immunohistochemistry to estimate the total number of the actively dividing cells. In addition, neuronal survival of the cells that have undergone maturation was evaluated by DAPI staining in TS hippocampus after chronic RO4938581 administration. Next, we used GAD65, GAD67 and VGAT immunostaining to evaluate whether RO4938581 chronic treatment affected the density of GABAergic synapses in the hippocampus of TS and CO mice.
Results: Chronic administration of RO4938581 improved synaptic plasticity and neurogenesis deficits in TS mice. In hippocampal slices from chronically treated animals, RO4938581 reversed the LTP deficit found in the CA1 region of the TS mice which is in agreement with the known distribution of GABA-A alpha 5 receptors. In addition, we found that RO4938581 treatment completely restored the number of Ki67+ cells in the DG of TS mice and produced a less pronounced enhancement in the density of this cell population in CO mice ($p = 0.033$). Neuronal survival of the cells that have undergone maturation was also normalized in TS mice as shown by the increase in DAPI+ cells found in TS hippocampus after chronic RO4938581 administration ($p = 0.026$). There was no significant increase in DAPI+ cells in RO4938581-treated CO mice. Furthermore, we found that RO4938581 chronic treatment affected the density of GABAergic synapses in the hippocampus of TS and CO mice. We found increased number of GAD65, GAD67 and VGAT positive boutons in the hippocampus of vehicle-treated TS mice compared to vehicle-treated CO mice ($p = 0.001$, in all cases). After chronic treatment with RO4938581, the percentage of area occupied by GAD65, GAD67 and VGAT positive boutons was significantly decreased in the hippocampus of TS mice ($p = 0.017$, $p = 0.016$ and $p = 0.019$ respectively) similar to that observed in vehicle-treated CO mice.
Conclusions: These results indicate that reduction of GABA-A alpha 5 mediated inhibition by RO493858 restored synaptic plasticity, corrected deficient neurogenesis and normalized the density of GABAergic synapses in in the hippocampus of TS mice. These data further support the potential therapeutic use of selective GABA-A alpha 5 negative allosteric modulators to treat cognitive dysfunction in DS.
Keywords: Down syndrome, Ts65Dn, GABA-A alpha 5, Snaptic plasticity, Neurogenesis
Disclosure: P. Martinez, Nothing to Disclose; C. Martinez-Cue, **Part 4:** Received a research grant from F. Hoffmann-La Roche Ltd; N. Rueda, Nothing to Disclose; R. Vidal, Nothing to Disclose; S. Garcia, Nothing to Disclose; V. Vidal, Nothing to Disclose; A. Corrales, Nothing to Disclose; J. Montero, Nothing to Disclose; A. Pazos, Nothing to Disclose; J. Florez, Nothing to Disclose; A. Thomas, **Part 4:** Employed by F- Hoffmann-La Roche; F. Knoflach, **Part 4:** Employed by f. Hoffmann-La Roche Ltd; J. Trejo, Nothing to Disclose; J. Wettstein; M. Hernandez, **Part 4:** F. Hoffman-La Roche Ltd is my employer.

M94. Glutamate Antagonism and Alcohol Behaviors in Heavy Drinkers: Contrasting Effects of Opioid Antagonism

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Background: Ethanol's rewarding properties are mediated through the brain corticostriatal pathway and glutamate and opioid neurotransmitters play important roles in modulating this pathway. The NMDA glutamate receptor is one of the primary targets for ethanol in the brain, and NMDA receptor antagonists reduce operant responding for alcohol and alcohol self-administration. However, clinical evidence on the influence of NMDA antagonists like memantine on alcohol drinking behaviors remains controversial. Two open-label trials with memantine, at a dose of 20 mg/day, reported improvement in alcohol-related dementia (Cheon et al., 2008) and reduction in alcohol drinking and urges to drink (Arias et al., 2007) while a clinical trial of a 40 mg/day dose in alcohol-dependent drinkers did not demonstrate any significant benefits (Evans et al., 2007). To further understand these discrepant findings, we evaluated the effects of memantine (MEM) on alcohol drinking behaviors in heavy drinkers. Additionally, considering the proposed role for family history (FH) of alcoholism on responses to glutamatergic agents (Petrakis et al., 2004) we also evaluated the influence of FH status. In order to delineate the roles of gluatamate versus opioid systems in alcohol drinking behaviors, we compared and contrasted these results with our earlier work where we examined the interactive effects of FH status and naltrexone dose on drinking behaviors (Krishnan-Sarin et al., 2007).
Methods: 120 non-treatment seeking heavy drinkers, who were either FHP or FHN, consuming between 20-70 drinks/week, were randomly assigned to receive one of three doses of MEM (0, 20 or 40 mg/day) for a seven-day period. On the seventh day subjects participated in an Alcohol Drinking Paradigm (ADP; O'Malley et al., 2002). Specifically, following baseline monitoring subjects were exposed to a priming dose (PD) period during which they were provided with a PD (0.03 g/dl) and alcohol craving (Yale Craving Scale and Alcohol Urge Questionnaire) and stimulation/sedation (Biphasic Alcohol Effects Scale) were monitored every 10 minutes for 50 minutes. Participants were then exposed to three, one-hour self-administration (SA) periods, and during each hour they were offered the choice of consuming four drinks (0.015 g/dl each) or receiving $3 in exchange for each drink. Craving and alcohol-induced stimulation/ sedation were monitored every half hour. ANOVA's and correlational analyses were used to evaluate the influence of MEM dose and FH status on the primary (alcohol craving and number of drinks consumed) and secondary (alcohol-induced stimulation and sedation) outcomes.
Results: 90 heavy drinkers (45 FHP, 45 FHN) completed this study. When examining the PD period alone, we observed a significant MEM effect ($p < 0.05$) on changes in alcohol craving from baseline to end of PD; specifically, alcohol craving was reduced in participants who received the 20 mg/day of memantine when compared with the 0 and 40 mg/day doses. Pos-hoc tests indicate that this effect was primarily observed in FHP and not in FHN heavy drinkers. We also observed a MEM effect ($p < 0.05$) on alcohol-induced stimulation during the PD period with post-hoc analyses indicating that alcohol-induced stimulation was reduced by the 20 mg/day dose (when compared with the 0 and 40 mg/day doses). When examining the SA periods, we did not observe significant effects of memantine on number of drinks consumed. However, MEM at 20 mg/day continued to significantly suppress alcohol-craving and alcohol- stimulation.
Conclusions: These results suggest that memantine's effects are dependent on dose, family history status as well as the type of alcohol behaviors being examined. We observed that memantine

reduced alcohol craving and stimulation without influencing actual drinking. Interestingly, and in contrast to these findings, we have previously shown that the opioid antagonist naltrexone reduced drinking in FHP but not FHN drinkers (Krishnan-Sarin et al., 2007) without producing any changes in alcohol craving or alcohol-induced stimulation/sedation (unpublished findings). Taken together, these studies provide the first demonstration of distinctive roles for the glutamatergic and opioidergic systems in FHP drinkers; specifically, blocking opioid receptors with naltrexone appears to reduce stimulus response habits and alcohol drinking, while blocking NMDA receptor function with memantine appears to reduce alcohol reward. (Supported by P50AA12870)
Keywords: Alcohol Drinking, Alcohol Craving, Memantine, Naltrexone
Disclosure: S. Krishnan-Sarin, **Part 4:** I am the PI on a multiste Chantix trail in adolescent smokers being conducted by Pfizer. I have also received a GRAND award from Pfizer to evaluate neurocognitive correlates of Chantix treatment in adolescent smokers; S. O'malley, **Part 1:** Member of the Alcohol Clinical Trial Initiative, sponsored by Abbott, Eli Lilly, Johnson & Johnson, Schering Plough, Lundbeck, GlaxoSmithKline and Alkermes, Contract, Nabi Biopharmaceuticals, Medication supplies, Pfizer Inc, Advisory Board, Gilead Pharmaceuticals, Lundbeck, Consultant, GlaxoSmithKline, Pfizer; N. Franco, Nothing to Disclose; D. Cavallo, Nothing to Disclose; B. Pittman, Nothing to Disclose; J. Shi, Nothing to Disclose; J. Krystal, **Part 1:** Consultant, Note: – The Individual Consultant Agreements listed below are less than $10,000 per year, Aisling Capital, LLC, Astellas Pharma Global Development, Inc., AstraZeneca Pharmaceuticals, Biocortech, Brintnall & Nicolini, Inc., Easton Associates, Gilead Sciences, Inc., GlaxoSmithKline, Janssen Pharmaceuticals, Lundbeck Research USA, Medivation, Inc., Merz Pharmaceuticals, MK Medical Communications, F. Hoffmann-La Roche Ltd, Sage Therapeutics, Inc., SK Holdings Co., Ltd, Sunovion Pharmaceuticals, Inc., Takeda Industries, Teva Pharmaceutical Industries, Ltd., Scientific Advisory Board, Abbott Laboratories, Bristol-Myers Squibb, CHDI Foundation, Inc., Eisai, Inc., Eli Lilly and Co., Forest Laboratories, Inc., Lohocla Research Corporation, Mnemosyne Pharmaceuticals, Inc., Naurex, Inc., Neurobiology Foundation-Research in Schizophrenia and Bipolar Disorder, Pfizer Pharmaceuticals, Shire Pharmaceuticals, StratNeuro Research Program at Karolinska Institute (International Advisory Board), Board of Directors, Coalition for Translational Research in Alcohol and Substance Use Disorders, President, American College of Neuropsychopharmacology, Editorial Board, Income Greater than $10,000, Editor - Biological Psychiatry, Employment, Yale University School of Medicine, VA CT Healthcare System, Patents and Inventions 1) Seibyl JP, Krystal JH, Charney DS. Dopamine and noradrenergic reuptake inhibitors in treatment of schizophrenia. Patent #:5,447,948. September 5, 1995, 2) I am a co-inventor with Dr. Gerard Sanacora on a filed patent application by Yale University related to targeting the glutamatergic system for the treatment of neuropsychiatric disorders (PCTWO06108055A1), 3) Intranasal Administration of Ketamine to Treat Depression (pending).

M95. Occupancy of Naltrexone at Kappa Opioid Receptors May Predict Efficacy in Reducing Craving and Drinking in Alcoholics: A PET Imaging Study with a Novel Kappa Tracer

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Background: Naltrexone (NTX) is a non-selective opioid antagonist that has been shown to have variable efficacy in treating alcohol addiction. It is known that an individual's family history of alcoholism is a predictor of positive response to NTX (e.g. Krishnan-Sarin *et al.*, 2007). The importance of NTX's binding at one or another opioid receptor site (mu, kappa, or delta) in relationship to its efficacy is not well understood. It has been shown previously, with PET and ^{11}C-carfentanil, that a single 50 mg dose of NTX causes almost complete occupancy of the Mu-opioid receptors (MOR) in the brains of alcoholics (Weerts *et al.*, 2008). This observation offers a hint that any differential efficacy of NTX between alcoholics (shown by others) is related to binding at sites *other* than Mu. Thanks to the development of a new kappa tracer, we have been able to study the Kappa-binding of NTX in alcoholics. The present pilot project is the first study to explore the relationship between naltrexone occupancy at the kappa opioid receptors (KOR) and naltrexone-induced changes in drinking behavior in alcohol-dependent heavy drinkers.
Methods: We examined the relationship of KORs and and NTX occupancy of KOR in non-treatment seeking, alcohol dependent (SCID; First et al., 1996), heavy drinkers (20-70 drinks for women and 25-75 drinks for men; TLFB, Sobell and Sobell, 1992) with a positive and negative family history of alcoholism (FHAM; Rice et al., 1995.) KOR binding was measured using Positron Emission Tomography (PET) Imaging with our new selective KOR-antagonist tracer, ^{11}C-LY2975050 (Zheng *et al.*, submitted). PET imaging was performed before and after a week to 10 days of NTX treatment on the Siemens High Resolution Research Tomograph (HRRT). Arterial blood was collected and metabolite corrected plasma samples were used for input function estimation. Scans were 90 minutes long. A 2 tissue-compartment model was fitted to time-activity curves at the region-of interest level to estimate regional volumes of distribution, V_T, of the tracer. V_T is a measure of receptor density plus background uptake of tracer but in the absence of a reference region (region devoid of Kappa receptors), it is the most reliably estimated index of specific tracer binding. V_T values prior to treatment may reflect in-born differences in KOR between subjects or effects of years of drinking. Modified occupancy plots (Cunningham et al., 2010) were constructed using V_T, atbaseline and after NTX, to estimate a brain-wide occupancy of KOR by NTX after the treatment period. Naltrexone-induced changes in drinking behavior were determined using an Alcohol Drinking Paradigm (ADP; O"Malley et al., 2000; Krishnan-Sarin et al., 2007). All subjects participated in two ADP sessions – one at baseline before NTX treatment and one after 6-11 days NTX exposure. The dose of NTX was tapered up to 100 mg/day over the first 3 days of this treatment period. Each ADP session was initiated with a priming drink which had to be consumed. Subsequently, subjects were exposed to three, one-hour choice periods during which they were given the choice of consuming 4 alcoholic drinks or receiving $3 for each drink not consumed. During each ADP, craving for alcohol was measured using the Alcohol Urge Questionnaire (AUQ; Bohn et al., 1995) and the Yale Craving Scale (YCS).
Results: First, the occupancy of NTX at KOR varied much more widely [20% to 100%] within our preliminary cohort of alcohol-dependent subjects ($n = 6$) than did NTX occupancy at MOR as imaged with ^{11}C-CFN (Weerts et al; 2008). Second, we defined a low occupancy group (less than 90% occupancy) and a high occupancy group ($>90\%$). The high occupancy group had greater reduction (ΔCrave) in craving on the YCS ($\Delta Crave = 10.25 \pm 6.60$ vs. 4 ± 5.66) as compared to the low occupancy group. The high occupancy group also experienced a greater reduction in their drinking (ΔDrinks) -as measured by the two ADP sessions - as compared to the low occupancy group ($\Delta Drinks = 5 \pm 2.94$ drinks vs. 2.5 ± 2.12 drinks). Third, the high occupancy group ($n = 4$) was made up entirely of subjects with a positive family history (FH) for alcoholism whereas the low occupancy group was made up of two alcoholics ($n = 2$) who were both negative for FH. We also examined the tracer V_T prior to treatment and found a trend

toward lower V_T (and by implication, lower KOR) in subjects who consumed more drinks in the baseline ADP.
Conclusions: Our preliminary data suggest that KOR may be related to drinking (as measured by the baseline ADP) and may play a key role in the action of NTX to help alcohol-dependent subjects reduce their craving and reduce their drinking (as measured by the post-treatment ADP). The differences seen between high-occupancy and low occupancy groups and their respective decreases in craving and drinking from before to after NTX could help to explain the differential efficacy (shown previously) for NTX in FH positive and FH negative populations of alcoholics. (Supported by P50AA12870)
Keywords: Kappa receptor, naltrexone, alcohol drinking, alcohol craving, neuroimaging
Disclosure: E. Morris, Nothing to Disclose; S. Kim, Nothing to Disclose; N. Franco, Nothing to Disclose; D. Cavallo, Nothing to Disclose; A. jordan, Nothing to Disclose; J. Gillard, Nothing to Disclose; M. Zheng, Nothing to Disclose; S. lin, Nothing to Disclose; S. O'malley, **Part 1:** Member of the Alcohol Clinical Trial Initiative, sponsored by Abbott, Eli Lilly, Johnson & Johnson, Schering Plough, Lundbeck, GlaxoSmithKline and Alkermes, Contract, Nabi Biopharmaceuticals, Medication supplies, Pfizer Inc, Advisory Board, Gilead Pharmaceuticals, Lundbeck, Consultant, GlaxoSmithKline, Pfizer; Y. Huang, Nothing to Disclose; S. Krishnan-Sarin, **Part 4:** Yale PI on multiste adolscent Chantix trial conducted by Pfizer, Neuroimaging study of Chantix in adolescent smokers funded by GRAND award from Pfizer.

M96. Development of Personalized Small Molecule Modulator Screening Strategies: Upregulation of Alpha-L-iduronidase in Mucopolysaccharidosis Type I (MPSI) Patient Cells

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Background: Mucopolysaccharidosis type I (MPSI; Hurler disease) is an autosomal recessive disorder caused by deficiency of the lysosomal glycosaminoglycan-degrading enzyme α-L-iduronidase (IDUA). More than 100 disease-causing mutations have been reported in the IDUA gene, resulting in a wide range of phenotypes including: mental retardation, motor dysfunction, skeletal deformities, and spleen and liver enlargement, among others. Enzyme replacement therapy can successfully treat a number of symptoms, notably excluding mental retardation due to the lack of the ability of the exogenous enzyme to enter the brain; a small molecule approach is therefore highly attractive. There are no small molecule drugs currently available to treat MPSI patients. Here, we employed a screening strategy to upregulate enzyme activity in specific patient cells, a method that has applications for personalized screening to identify patient specific treatment regimens for IDUA upregulation.
Methods: We developed two screening strategies employing patients' skin fibroblasts in order to identify compounds capable of increasing IDUA gene expression and IDUA enzymatic activity. For the gene expression assay, we miniaturized Cells-to-Ct technology followed by high-throughput real time PCR for Taqman-based real-time quantification of IDUA gene expression thus enabling 384-well based compound screening. For the enzymatic activity assay, 4-Methylumbelliferyl alpha-L-iduronide substrate was utilized, which, when cleaved by IDUA, liberates the fluorescent molecule 4-Methylumbelliferone. Increased IDUA enzyme activity is therefore correlated with increased well fluorescence.
Results: IDUA enzymatic activity and gene expression assays were high-throughput screening (HTS) amenable with Z-factor values greater than 0.5, allowing screening of several small molecule libraries. Hit rates were ~3% with gene expression and ~0.8% for IDUA enzymatic activity assays, suggesting a slightly higher level of sensitivity in the enzymatic assay. As confirmation of higher sensitivity, less than 50% of the hits identified in the gene expression assay were confirmed for effects on IDUA activity, while 100% of the hits found in the IDUA enzymatic activity assay were confirmed at the dose response stage.
Conclusions: Our unpublished data show that the enzymatic activity assay we developed for small molecule screening is simple, inexpensive and has many characteristics of a suitable assay for routine identification of active small molecules using patient derived cells. The libraries selected for these efforts include natural compound libraries as well as FDA-approved drug libraries from which identified lead compounds with known safety profiles can be repositioned in a relatively short amount of time. Importantly, these efforts were performed on cells taken directly from the patient, a strategy that can be expanded to include a greater number of patients with a variety of different mutations. These principles can also be applied to other types of lysosomal storage diseases such as Gaucher's and Tay-Sachs diseases.
Keywords: IDUA, Mucopolysaccharidosis, drug dcreening, lysosomal disorders, small molecules
Disclosure: C. Volmar, Nothing to Disclose; S. Brothers, Nothing to Disclose; C. Wahlestedt, **Part 4:** Opko Health.

M97. Trajectories of Depressive Symptoms During Medical Internship: Insights Into Classes of Depressive Symptoms Under Conditions of Chronic Stress

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Background: To assess the presence of trajectory classes of depressive symptoms in response to chronic stress and identify predictors of class membership.
Methods: Medical internship was used as a prospective stress model. Interns from US residency programs completed online surveys, assessing demographic and psychological characteristics and depressive symptoms two-months prior to internship and at 3-month intervals throughout internship year. Depressive symptoms were measured via the nine-item Patient Health Questionnaire. Growth mixture modeling was used to identify and characterize trajectory classes.
Results: 2,278 (59%) of interns chose to take part in the study. Three classes of depressive symptoms were identified: 1) Stress-Resilient Class: 62% of participants report low depressive symptoms before and throughout internship year; 2) Stress-Neutral Class: 22% of participants report mild depressive symptoms before and throughout internship year; and 3) Stress-Sensitive Class: 16% of participants report low depressive symptoms before internship stress, and high levels of depressive symptoms throughout internship year. Individuals in the Stress-Sensitive class were more likely to be female, in a surgical specialty, and have a history of depression, difficulty early family environment and high neuroticism scores compared to individuals in the Stress-Resilient class.
Conclusions: Trajectory based analysis allows for the identification of a small high-risk group, within a heterogeneous population, that accounts for the link between stress and depression. Our study provides evidence of distinct classes of depressive symptoms in response to chronic stress and identifies a set of pre-stress predictors of class membership, a finding with significant implications for clinical intervention.
Keywords: Residency, Depression, Growth, Modeling
Disclosure: S. Sen, Nothing to Disclose; C. Guille, Nothing to Disclose; S. Clark, Nothing to Disclose; A. Amstadter, Nothing to Disclose.

M98. Lurasidone Adjunctive to Lithium or Valproate for the Treatment of Bipolar I Depression: Results of the 6-Week, Double-blind, Placebo-controlled Prevail-1 Study

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Background: Currently, first-line treatment of bipolar disorder consists of treatment with divalproex or lithium (APA-2002). However, the response of bipolar depressive states to treatment with these drugs is often incomplete, requiring adjunctive treatment (Bauer-1999, Calabrese-2003). While combination treatment, specifically mood stabilizers plus antidepressants or atypical agents (or both) is common in bipolar disorder, it is not clear whether agents with bipolar antidepressant effects should be primarily used as monotherapy or as adjunctive to other agents The goal of this study was to evaluate the efficacy and safety of lurasidone, adjunctive to lithium or valproate, in patients with bipolar I depression, without psychotic features.
Methods: In this multi-regional study, subjects ($n = 346$) meeting DSM-IV-TR criteria for bipolar I depression with a Montgomery-Åsberg Depression Rating Scale (MADRS) score ≥ 20, were randomized to 6 weeks of double-blind (DB) treatment with either lurasidone 20-120 mg/day (LUR) or placebo (PBO), both adjunctive to either lithium (Li) or valproate (VPA). Therapeutic blood levels of Li or VPA were maintained for ≥ 28 days prior to randomization. Changes from DB baseline (DB BL) in MADRS (primary assessment), and secondary efficacy outcomes were analyzed using either mixed model repeated measures (MMRM) or analysis of covariance, last observation carried forward (ANCOVA-LOCF), or logistic regression.
Results: Overall, 78% of LUR- (143/183) and 83% of PBO-treated subjects (136/163) completed the study. Mean MADRS scores at DB BL were similar for LUR (30.6) and PBO (30.8), indicative of moderate-to-severe depression. At Week 6, LUR was associated with a significantly greater MADRS reduction vs. PBO (-17.1 vs. -13.5; $p < 0.01$; MMRM; effect size, 0.30). Similarly, LUR treatment reduced CGI-bipolar severity (CGI-BP-S) depression ratings by -2.0 vs. −1.5 for PBO ($p < 0.01$; MMRM; effect size, 0.36), and improved Sheehan Disability Scale (SDS) total scores by −9.5 vs. −7.0 for PBO ($p \leq 0.01$; ANCOVA-LOCF). Significant improvements vs. PBO were also observed for anxiety symptoms, assessed by the HAM-A total score (−8.0 vs. −6.0; $p < 0.01$; ANCOVA-LOCF), and in quality of life, assessed by the Quality of Life, Enjoyment and Satisfaction Questionnaire (Q-LES-Q-SF; +22.2 vs. +15.9; $p < 0.01$; ANCOVA-LOCF). Responder rates (reduction in MADRS $\geq 50\%$) were significantly higher for the LUR (57%) than for the PBO group (42%; $p < 0.01$). Discontinuation rates due to adverse events were 6% for LUR and 8% for PBO. Most frequently reported adverse events were nausea (17.5% vs. 11.0%), headache (10.4% vs. 12.3%), and somnolence (8.7% vs. 4.3%) for LUR vs. PBO, respectively.
Conclusions: In this study, adjunctive use of lurasidone compared with placebo significantly improved depressive symptoms in patients with bipolar I depression who had inadequate response to either Li or VPA alone. Lurasidone treatment also significantly improved measures of functioning and quality of life. The low discontinuation rate due to adverse events, and low incidence of adverse events, suggest that lurasidone was well-tolerated as an adjunctive therapy in this study.
Keywords: bipolar disorder major depressive disorder Lorasidone lithium valproate
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M99. Static and Dynamic Functional Network Connectivity during Resting State in Schizophrenia

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Background: Schizophrenia has been associated with disrupted brain connectivity among distant brain regions. Intrinsic connectivity networks (ICNs) obtained using functional connectivity analysis of resting-state functional magnetic resonance imaging (fMRI) data seem to provide valuable insights in understanding the properties of these networks. In this study, we explore whole brain resting-state connectivity differences between healthy controls (HC) and patients with schizophrenia (SZ) who took part in the FBRIN Phase III multi-site fMRI study. In particular, we investigated both static and dynamic properties of functional network connectivity (FNC), defined as pairwise correlation between the time courses of ICNs.
Methods: Six minute resting-state fMRI scans were obtained from 163 healthy controls and 151 age-and gender-matched patients with SZ on seven 3 Tesla scanners across the US. A high model order ($C = 100$) group independent analysis was performed using all healthy control and patient resting fMRI data. Out of the 100 components, 47 ICNs were identified visually based on their spatial extent and the presence of greater low frequency power in their corresponding spectra. Subject-specific ICNs were obtained using a back reconstruction approach as implemented in GIFT software. Each subject's time courses were then detrended, orthogonalized to subject motion, and bandpass filtered between [0.01-0.15] Hz. Static FNC was computed between each ICN pair for each subject. Two-sample *t*-tests were performed on the FNC scores between groups after the correlation values were converted to z-scores using Fisher-Z transformation. Additionally, instead of assuming stationarity in network connectivity between ICNs during the whole scan duration, we estimated dynamic FNC by computing the pairwise correlations between ICNs in a sliding windowed fashion

(44 s window length) with an additional sparsity constraint on the inverse covariance matrix. These dynamic FNC states were then clustered using *k*-means clustering.
Results: The identified 47 ICNs were broadly categorized into 8 sub-networks: subcortical, auditory, sensorimotor, visual, default-mode, higher order associative, frontal, and cerebellar networks. The static FNC difference maps between HC and SZ revealed significant reductions in within network connectivity between subcortical, auditory, sensorimotor, and visual networks in patients with SZ, hyperconnectivity within default-mode network regions of patients with SZ, as well as reductions in anti-correlations between subcortical-auditory, subcortical-sensorimotor, subcortical-visual, and sensorimotor -visual connectivity. *K*-means clustering of dynamic FNC states revealed similar centroid FNC states for both HC and SZ subjects for several cluster sizes searched ($K = 2$ to 9), but the FNC window states of patients with SZ were more commonly assigned to a particular state whereas HC switched between states more often. The static FNC differences are primarily driven by these dynamic states to which SZ subjects are less likely to switch to. Furthermore, at $K = 9$, few patients with SZ exhibit a highly connected state across most ICNs suggesting aberrant wiring among brain networks.
Conclusions: Whole brain static FNC analysis results are consistent with earlier reports of increased hyperactivity in default-mode regions in patients with SZ. A default-mode regions hyperactivity might contribute to a relative failure to suppress these regions during active conditions and result in decreased performance in demanding tasks. Also altered sub-cortical and sensory (auditory, motor and visual) network connectivity in patients with SZ is possibly associated with hallucinations. Dynamic FNC analysis suggests that patients with SZ tend to linger in a state of "weak" and relatively "rigid" connectivity among different sub-networks. In contrast HC are probably faster in recruiting necessary resources as task demands change by dynamically switching between different FNC states while varying connectivity among sub-networks.
Keywords: schizophrenia; resting state; functional network connectivity; fmri; intrinsic connectivity networks
Disclosure: E. Damaraju, Nothing to Disclose; J. Turner, Nothing to Disclose; A. Preda, **Part 1:** Boehringer-Ingelheim Advisory Board, **Part 2:** University of California Irvine; T. Van Erp, Nothing to Disclose; D. Mathalon, **Part 1:** Consultant to BristolMyersSquibb Inc; J. Ford, **Part 1:** Bristol Myers Squibb; S. Potkin, **Part 1:** Bristol-Myers Squibb, Eisai, Inc., Eli Lilly, Forest Laboratories, Genentech, Janssen Pharmaceutical, Lundbeck, Merck, Novartis, Organon, Pfizer, Roche, Sunovion, Takeda Pharmaceutical, Vanda Pharmaceutical, **Part 2:** Lundbeck, Merck, Novartis, Sunovion, **Part 3:** Lundbeck, Merck, Novartis, Sunovion, **Part 4:** Amgen, Baxter, Bristol-Myers Squibb, Cephalon, Inc., Eli Lilly, Forest Laboratories, Genentech, Janssen Pharmaceutical, Merck, Novartis, Otsuka, Pfizer, Roche, Sunovion, Takeda Pharmaceutical, Vanda Pharmaceutical, NIAAA, NIBIB, NIH/NCRR, University of Southern California, UCSF, UCSD, Baylor College of Medicine; V. Calhoun, Nothing to Disclose.

M100. Effect of Narp Deletion on Neophobia

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Background: Neuronal activity regulated pentraxin, or Narp, is a secreted immediate early gene product that clusters AMPA receptors. We have previously shown that Narp knockout (KO) mice display extinction deficits in a morphine conditioned place preference paradigm. Studies suggest that both animals and humans who display addictive behavior also show increased novelty-seeking, although this association is complex and contradictory evidence exists. We sought to determine whether Narp KO mice, which display increased morphine craving, also display increased novelty-seeking.
Methods: To assess reactivity to novelty, Narp KO mice and littermate wild-type (WT) controls were tested on a neophobia paradigm. Firstly, mice were singly housed and habituated to a liquid rodent diet over 1 week that was available ad libitum. The following week, presentation of the diet was restricted to two hours in the morning and one hour in the afternoon. On days 3, 5 and 8, instead of receiving the liquid diet over one hour in the afternoon, mice were presented with a 3% decaffeinated coffee solution.
Results: Both Narp KO and WT control mice demonstrated neophobia to the coffee solution but did not significantly differ in their consumption of the coffee solution at initial exposure on day 3. However, Narp KO mice demonstrated impaired recovery from neophobia, drinking less than WT mice on exposure to the coffee solution on day 5 ($p < 0.01$, paired Student's t-test) and day 8 ($p < 0.005$, paired Student's t-test).
Conclusions: Our data indicate Narp KO mice avoid novelty rather than displaying the typical novelty-seeking behavior of addiction-prone mice. These results suggest that addiction may co-occur with novelty-avoidant behavior. Furthermore, the results also suggest that recovery from neophobia may utilize similar neurobiological pathways as extinction learning and that these pathways involve Narp.
Keywords: extinction learning, novelty, addiction, immediate early gene
Disclosure: A. Blouin, Nothing to Disclose; J. Lee, Nothing to Disclose; B. Tao, Nothing to Disclose; A. Johnson, Nothing to Disclose; D. Smith, Nothing to Disclose; J. Baraban, Nothing to Disclose; I. Reti, Nothing to Disclose.

M101. Combined Dexamethasone Suppression – Corticotrophin-releasing Hormone Stimulation Test in Unmedicated Major Depression and Healthy Volunteers

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Background: The HPA axis is dysfunctional in a subgroup of mood disorders. We compared cortisol and ACTH levels in depressed patient and healthy controls using the combined dexamethasone suppression – corticotrophin-releasing hormone stimulation (DEX-CRH) test. The uniqueness of our study is that the patients were medication-free and the controls did not have a history of a mood or psychotic disorder in their first-degree relatives. This is important because abnormal cortisol reactivity has been reported in individuals without psychopathology but with first-degree relatives with mood disorders.
Methods: Forty-two subjects participated in the study: 17 patients with major depressive disorder (MDD) and 25 healthy volunteers. Demographic and clinical parameters were assessed and recorded. Participants received an oral dose of 1.5 mg dexamethasone at 11 pm the day before the CRH administration. On the following day, at 3 pm, 100 μg of ovine CRH was infused. Blood samples from which cortisol and ACTH were determined were collected from 3 pm to 4:15 pm every 15 min. Cortisol and ACTH responses were calculated as areas under the curve.
Results: We found no difference between cortisol baseline levels (i.e., post-dexamethasone) in patients and controls before and after controlling for age. We also observed no difference in cortisol responses in patients and controls including after controlling for age and for age and baseline cortisol levels. Controlling for age, baseline (i.e., post-dexamethasone) ACTH levels were significantly higher in depressed patients compared to controls ($F = 8.96$, $p = 0.01$). There was a trend towards higher ACTH responses in depressed patients compared to the control group ($F = 4.03$,

$p = 0.08$). In depressed patients, cortisol and ACTH responses positively correlated with age ($r = 0.63$, $p = 0.007$, and $r = 0.65$, $p = 0.005$, respectively), the length of illness ($r = 0.49$, $p = 0.04$, and $r = 0.55$, $p = 0.02$, respectively), and the number of hospitalizations ($r = 0.93$, $p = 0.001$, and $r = 0.98$, $p < 0.001$, respectively).
Conclusions: Elevated baseline levels of ACTH in depressed patients observed in our study suggest that the feedback inhibition of resting ACTH secretion by cortisol is impaired in depressed patients. A trend towards higher ACTH responses to CRH administration in depressed patients compared to healthy controls indicates that depressed patients have better capacity to escape dexamethasone suppression compared to the control group. Our results do not support the hypothesis that elevated cortisol response to DEX-CRH test is a biomarker for MDD diagnosis, but instead suggest that the feedback inhibition of ACTH secretion by cortisol is compromised in MDD.
Keywords: Dexamethasone suppression – corticotrophin-releasing hormone stimulation test, cortisol, ACTH, depression
Disclosure: L. Sher, Nothing to Disclose; M. Oquendo, Nothing to Disclose; T. Cooper, Nothing to Disclose; J. Mann, Nothing to Disclose.

M102. Inflammatory Biomarkers in Late-life Depression and White Matter Integrity

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Background: Several lines of evidence have implicated inflammatory pathways in major depressive disorder (MDD). These include reports of elevations in various pro-inflammatory cytokines including interleukin-6 (Il-6), interleukin-8 (IL-8), interleukin-1β (IL-1 β). These cytokine elevations have also been reported in late-life depression and linked both to greater severity of baseline depressive symptoms and incident depression. A major drawback of existing cytokine studies is that they have been limited to determinations in plasma. Since these large molecules do not usually cross the blood-brain barrier, their relevance to neuroinflammation is not known. Most of these studies have also not included a neuroimaging component. This is particularly surprising, since white matter abnormalities implicated in depression may be especially sensitive to neuroinflammation. We examined a number of cytokines in both CSF and plasma in elderly individuals with MDD and healthy controls and hypothesized that elevations in certain cytokines would be found in MDD and would have a negative effect on measures of white matter integrity as determined by Diffusion Tensor Imaging (DTI).
Methods: To test this hypothesis, IL-6, IL-8, IL-1 in were measured in CSF and plasma in 29 older subjects with MDD and 19 controls. MRI scans were performed to rule out structural brain abnormalities and to assess fractional anisotrophy (FA), a measure of white matter integrity. All had intact cognition (no dementia and a Mini-Mental State Exam score of at least 28) and no gross MRI abnormalities other than white matter hyperintensities.
Results: Contrary to our prediction, there was no significant group difference in any of the CSF cytokines levels that we examined. However, consistent with previous reports, plasma IL-8 was elevated in individuals with MDD. Interestingly, in the entire sample, plasma IL-8 was negatively correlated with mean brain FA ($r = -0.307$; $p = 0.36$).
Conclusions: We found no evidence of CSF cytokine abnormalities in elderly subjects with MDD. However, there was an increase in plasma IL-8 in MDD, which merits further study because of its possible association with white matter pathology.
Keywords: late-life depression, pro-inflammatory biomarkers, white matter integrity, DTI, interleukins
Disclosure: N. Pomara, Nothing to Disclose; D. Bruno, Nothing to Disclose; J. Nierenberg, Nothing to Disclose; J. Sidtis, Nothing to Disclose; H. Zetterberg, Nothing to Disclose; K. Blennow, Nothing to Disclose.

M103. Acute Nicotine Administration Improves White Matter Integrity and Associated Attention Performance

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Background: Ligand binding studies demonstrate that, in addition to being expressed on synaptic terminals, nicotinic acetylcholine receptors (nAChRs) are expressed in the cerebral white matter (WM). We tested the hypothesis that an acute nicotine pharmacological challenge will alter WM integrity measured as change in the fractional anisotropy (FA) and that this change is predictive of cognitive effects of nicotine.
Methods: A randomized, nicotine patch vs. placebo patch, crossover, double-blind clinical trial. Adult smokers ($N = 39$, 20 controls and 19 schizophrenic patients) were evaluated by diffusion tensor imaging under nicotine vs. placebo patches. Findings of significant nicotine-related FA change were tested in a replication cohort of healthy adult smokers ($N = 38$). Average FA values for the whole brain and nine preselected WM tracts were calculated using tract-based-spatial-statistics. Nicotine-related FA changes were examined in relationship to behavioral performance of a sustained attention/working memory task.
Results: Nicotine-placebo difference in FA values for the genu of corpus callosum (DFA_{genu}) was significantly associated with average cotinine level (R^2 change = 35%, $p = 0.001$), a measure of nicotine metabolites from primarily recent smoking; a similar finding was observed in the replication cohort (R^2 change = 13%, $p = 0.02$). Smokers with lower serum cotinine levels were more likely to show a positive DFA_{genu} change in response to nicotine administration. Further, increased FA in the genu from placebo to nicotine patch explained 22% of variance in performance of a rapid visual sustained attention task during the nicotine session ($p = 0.006$).
Conclusions: We observed and replicated an acute pharmacological influence of nicotine on WM that appeared in part contingent upon recent nicotine intake and metabolism from recent smoking. Furthermore, the DFA_{genu} was significantly and positively correlated with nicotine-related improvement in sustained attention/working memory. The biophysical underpinning of the nicotine-related changes in FA signal is unknown, but could be due to stimulation of axonal, non-synaptic nAchRs that leads to alterations in local axonal extracellular environment and improvement in cerebral connectivity and cognitive performance.
Keywords: nicotine, DTI, white matter
Disclosure: P. Kochunov, Nothing to Disclose; E. Stein, Nothing to Disclose; E. Hong, Nothing to Disclose.

M104. The Unreliability of Reliability Statistics: A Primer on Calculating Interrater Reliability in CNS Clinical Trials

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Background: Clinical trial failure rates in clinical trials of known effective antidepressants and anxiolytics exceed 50% even when theoretically powered at 80–90%(Khin et al., 2011). However, power calculations rarely take into account the less than perfect reliability of subjective assessments in CNS clinical trials. Inaccurate or inflated reliability estimates can have substantial implications for not only study power but also sample size requirements and the ability to detect drug-placebo separation.

For example, a drop in reliability from .90 to .70 results in a decrease in power from 76% to 65% (Muller & Szegedi, 2002). The sample size to maintain power with this decrease in reliability would need to be increased 30% (Perkins, Wyatt, & Bartko, 2000). Decisions about further development of drug candidates require accurate estimates of reliability. Poor reliability or inappropriate reliability statistics can have significant consequences ranging from increased R&D costs to significant delays in getting effective drugs to patients who need them. Despite the importance of reliable outcome assessments, clinical trial reporting seldom includes estimates of interrater reliability (IRR; Mulsant et al., 2002). When reported, selection of reliability statistics is inconsistent and often inappropriate for the level of measurement or methodology employed. A set of guidelines is proposed for the appropriate selection of reliability measures for CNS clinical trials.
Methods: Commonly-used reliability statistics are reviewed and the appropriateness of their use with various data types and methodologies typical of CNS clinical trials is evaluated. Common misuses of reliability statistics are illustrated using a sample data set and the impact of inappropriate analyticselection on estimates of reliability is demonstrated.
Results: *Diagnosis:* Cohen's kappa is the most commonly used measure of IRR for psychiatric diagnosis (Cohen, 1960). Kappa can be used with binary, nominal or ordinal data with 2 or more raters rating 2 or more subjects. *Outcome Measurement:* Common efficacy outcomes in CNS clinical trials are summed total or subscale scores on psychiatric rating scales (e.g., MADRS, PANSS). Multiple measures of IRR for continuous measures are discussed. One measure to assess agreement between 2 or more raters is the paired-sample t-test or one-way repeated measures ANOVA. This test examines whether differences in scores between raters are statistically significant but does not estimate the degree of agreement between raters. Alternatively, the Bland-Altman test can be used to visually examine agreement between two raters on multiple observations (Bland & Altman, 1986). Intraclass correlation coefficients (ICC; Shrout & Fleiss, 1979) are appropriate for measuring IRR with continuous outcome measures when 2 or more raters rate 2 or more subjects. Decisions about which form of ICC is estimated should be based on the type and number of raters. Kappa has often been misused to estimate the IRR of continuous outcome measures. Continuous variables such as PANSS total score are sometimes dichotomized such that a fixed criterion (e.g., +/− 20%) is used to indicate rater agreement. Comparisons of ICC to Kappa using a 5, 10, and 20% criterion demonstrate that selecting a broader criterion range for Kappa can artificially inflate reliability estimates using the same data. Lin's Concordance Coefficient has been suggested as an alternative to the ICC to measure agreement between raters on multiple observations (Lin, 1989). The above statistics require that reliability be measured on more than 1 subject. However, it is not always possible to obtain multiple observations. In cases where 2 or more raters rated a single subject (as in a group calibration at an investigator meeting) it is possible to estimate interrater agreement (though not reliability). The most straightforward agreement statistic for a single observation is the Coefficient of Variation (COV). Alternatively, one can estimate r_{wg} (James, Demaree & Wolf, 1984) or average deviation (AD) indices (Burke, Finkelstein & Dusig, 1999). One common error is to treat individual items on a scale as independent observations. We demonstrate how ICCs calculated this way may actually be inversely related to reliability of a construct. Appropriate statistics for single observations may reveal IRR issues masked by this approach.
Conclusions: Reliability can have significant impact on clinical trial outcomes. Assessing IRR prior to study start enables researchers to design a methodology to fully exploit the strengths of a particular statistic. However, these estimates are often obtained without independent interviews (i.e., watching videotaped assessments), in artificial settings (i.e., at investigator meetings) and at a single point in time (i.e., prior to the start of the study). It is important to assess IRR of actual trial assessments throughout a study to evaluate rater drift. When selecting reliability statistics researchers must take into account the type of variable (e.g., binary, nominal, interval), the number of raters, composition of the rater pool (i.e., same raters rate all subjects vs. raters selected from a larger pool) and the number of observations. We demonstrate the impact of common misuses of reliability statistics on estimates of IRR.
Keywords: Methodology, statistics, agreement, reliability, kappa
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M105. Effects of Chronic Mild Stress and Electroconvulsive Seizure on Memory Functions in Rats

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Background: Chronic mild stress (CMS) is a valid rat model of depression. Chronic exposure to mild and unpredictable stressors induces an anhedonic-like state, which is monitored as a reduced intake of a sucrose solution. Anhedonic-like behavior can be reversed by chronic treatment with antidepressant drugs. In our hands, the CMS model has additional features that enhance its validity. Thus rats shown a graduated response to stress; a substantial fraction of animals submitted to stress are resilient and do not become anhedonia-like, but do have stress-induced cognitive impairments. Furthermore antidepressant administration reverses stress-induced anhedonia in approximately 50% of the treated animals, which mirrors clinical treatment refraction and is thus adding additional translational value to the CMS model. The purpose of the present study is to demonstrate consequences of the CMS paradigm on memory functions as well as addressing the effects on memory functions by acute and repetitive treatment with electroconvulsive shocks.
Methods: The CMS protocol was applied as described (1) i.e. rats were exposed to a number of different microstressors and sucrose intake was used as a read out of reward sensitivity i.e. hedonic status. Electroconvulsive seizure (ECS) modality was applied with pulse stimulator settings at 50 mA/0.5 s and repeated 10 times over 3 weeks. Memory functions were addressed by the spontaneous alternation behavior (SAB) test in the Y-maze and memory extinction was tested in the contextual fear conditioning test and in the step-down and step-through tests.
Results: We find an association between the anhedonia-like state and alterations in hippocampal-dependent memory (1) as well as for memory extinction. Thus anhedonic-like rats have a lower score in the SAB test, but a better performance in the extinction tests as compared to controls. In the SAB test there is a negative acute effect of ECS on controls when measuring performance 24 hrs after treatment. There is a robust effect of ECS on anhedonic-like rats with a response rate of 65% measured as a recovery of sucrose intake, and furthermore the responding rats also perform like controls in the SAB test.

Conclusions: On the basis of our investigations we conclude that the CMS model established in our laboratory has a substantial validity as a depression model thus mirroring anhedonia and inducing cognitive impairments. Cognitive disturbances include an improved memory function for negative events i.e. suggesting a cognitive bias or pessimistic-like behavior. The acute effect of ECS is demonstrated as a reduction in working memory, however, the chronic effect by repetitive treatments restores working memory functions, altogether very much similar to observations in clinical settings using electroconvulsive therapy. 1) Henningsen, K., Andreasen, J.T., Bouzinova, E.V., Jayatissa, M.N., Jensen, M.S., Redrobe, J.P., Wiborg, O. Cognitive deficits in the rat chronic mild stress model for depression: relation to anhedonic-like responses. Behavioural Brain Research, 2009, 198, 136-141.
Keywords: depression, ECT, antidepressant treatment, anhedonia, memory impairments
Disclosure: O. Wiborg, Nothing to Disclose; K. Henningsen, Nothing to Disclose; D. Woldbye, Nothing to Disclose; E. Bouzinova, Nothing to Disclose.

M106. Next-Generation Sequencing Follow-up to a Genome Wide Association Scan for EEG Power in a Native American Population

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Background: Electrical activity in the human brain, measured via the resting electroencephalogram (EEG), is trait-like, variable between individuals and associated with several psychiatric diseases including alcoholism, schizophrenia and anxiety disorders. Resting EEG power is substantially heritable however underlying functional variants are unknown. Genome-wide association for resting EEG power in a Plains Indian tribe identified a single genome-wide significant signal for alpha power on chromosome 12 (rs261900 $p = 2.5x10^{-8}$) in *BICD1*. Three other markers in LD with rs261900 also showed significant or sub-threshold associations to this trait. Three genome-wide significant signals were identified for theta power on chromosomes 1, 11 and 17. One of the regions on chromosome 1 generated a p-value for association of $5.13x10^{-9}$ at rs10889635 in the *SGIP1* gene, and this lies within a region previously linked to beta power in an earlier study (Ghosh et al 2003). The region on chromosome 17 (rs3333117, $p = 6.38x10^{-8}$) lies close to a previously linked marker (Saccone et al 2000), and identifies the *SPNS3* and *SPNS2* genes as candidates. No genome-wide significant peaks were identified for beta power, although several sub-threshold peaks were identified including a signal derived from a haplotype on chromosome 4 in the *GLRA3* gene. Only the association at *SGIP1* to theta power replicated in a North American Caucasian population, although the association at *BICD1* showed the same trend ($p = 0.054$). No functional variants that explain the observed phenotypes have been identified.
Methods: 24 unrelated Plains Indian subjects were selected on the basis of their haplotypes at *SGIP1*, *BICD1*, *UGDH* and *GLRA3* for exome sequencing to identify potentially functional variants that would result in the observed variation in EEG power. Exonic sequences were enriched using the Agilent SureSelect 50Mb human all-exon reagent which specifically targets protein coding sequences. Exome libraries were bar-coded, combined into two pools of 12 individuals and sequencing was performed using the Applied Biosystems 5500xl DNA analysis system. Pair-end sequence reads were mapped to human reference sequences (UCSC hg18) and SNPs were identified using LifeTech's LifeScope (version 2.5.0). The base counts were parsed for each SNP in each sample. SNPs' genic locations were identified based on RefSeq Genes from UCSC Human Genome hg18. The potential functional effects of missense cSNPs were predicted using PolyPhen (v2.2.2) and SIFT(v4.0.4). Information about known human SNPs from dbSNP Build 130 was added if there was a match.
Results: Sequencing 24 Plains Indians identified 173,976 SNP/SNVs present in 196,564 transcripts. Of these, 78,138 were not present in dbSNP v130 and are therefore novel. The observed transition/transversion ratio was 1.95, close to the expected value of 2, indicating a relatively low error rate of genotype calls. 412 nonsense variants were identified (308 novel) affecting 387 genes. Of these nonsense variants, 60% were observed only once in the dataset. Each individual had an average of 14 homozygous nonsense variants. A stop codon (Q173* - minor allele frequency 0.18) was observed in *GRK4* (G protein coupled receptor kinase 4), a protein that is associated with essential hypertension and desensitizes DRD1, DRD3, GABBR1 and GRM1 receptors. In addition other classes of functional variants were detected, missense (35,700), splice-site (7,426), promoter (1,208) and stop-loss (73). Analysis by haplotype failed to identify functional variants in *SGIP1*, *ST6GALNAC3*, *UGDH*, *BICD1* or *GLRA3*.
Conclusions: Exome sequencing in a relatively small Plains Indian sample identified multiple potentially functional variants. No variants were identified at *SGIP1*, *ST6GALNAC3*, *UGDH*, *BICD1* or *GLRA3* that explain the variation seen in EEG power. Known missense variants were detected in the *SGIP1* gene indicating that the failure to detect functional variants did not arise due to low coverage of the region, rather due to a failure to target the requisite genomic region. Newer exome enrichment bait libraries are now available which fully cover the 5′ and 3′-UTR sequences of the RefSeq genes and which will increase coverage of proximal promoter regions which may harbor functional variants that subtly alter gene expression levels. Although exome sequencing has failed in this instance to identify the functional variants underlying GWAS it has identified many functional variants within a Native American tribe, some of which appear to be restricted to this and closely related populations.
Keywords: Next-generation sequencing, Intermediate phenotype, Alcoholism, Genome-wide association study, functional polymorphism
Disclosure: C. Hodgkinson, Nothing to Disclose; P. Iarikova, Nothing to Disclose; Q. Yuan, Nothing to Disclose; C. Marietta, Nothing to Disclose; Z. Hommer, Nothing to Disclose; M. Enoch, Nothing to Disclose; D. Goldman, Nothing to Disclose.

M107. Extracellular Administration of Apical Domain of CCT1 Inhibits Mutant Huntingtin Aggregation and Promotes Cell Survival *in Vitro*

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Background: Studies have demonstrated that chaperonin-containing TRiC (CCT1) or its apical domain (ApiCCT1) suppresses huntingtin (Htt) aggregation and attenuates toxicity of abnormal Htt to cells and organotypic slices *in vitro*. Htt aggregation is promoted by exogenous application of aggregated protein seeds; in other words Htt aggregation is transmitted from cell to cell by seeding. Blocking the seeding-related Htt aggregation with ApiCCT1 has important implications as a potential therapy for Huntington's disease.
Methods: This study is designed to test whether extracellular aggregate seeding accelerates formation of Htt aggregates in cells and whether exogenous administration of ApiCCT1 can accordingly block this process and protect cells. Overexpression of green fluorescent protein (GFP)-tagged mutant Htt exon 1 protein (mHtt) that contains either 97 or 103 polyglutamine (polyQ) repeats (97Q-mHtt1-GFP or 103Q-mHtt1-GFP) is induced in RGC5 retinal ganglion cells following transient transfection or in 14A2.6 PC 12 cells following ponasterone A induction. Accumulation of mHtt aggregates is evaluated by both time-lapse fluorescence microscopy and biochemical gel analyses. Extracellular

administration of ApiCCT1 is achieved by transfection of a construct that secretes ApiCCT1. Oligomeric KKQ30KK, fibular 17aaQ40 and monomeric KKQ30KK seeding peptides were prepared by chemical synthesis.
Results: Fluorescent microscope quantification of cells with aggregates show a significant increase in the percentage of aggregate-positive cells at 16 hr post-seeding with 0.5-5.0 mM oligomeric KKQ40KK, compared with either 17aaQ40 (fibrils) or KKQ30KK (monomer). These results suggest that KKQ40KK oligomer seeding significantly promotes aggregation of cytoplasmic 97Q-Htt1-GFP in RGC5 cells. Similar results were also observed in 14A2.6 PC12 cells that express 103Q-mHtt-GFP following KKQ40KK aggregate seeding. 14A2.6 PC12 cells were treated with 2 ~ 5 mM ponasterone to induce expression of 103Q-mHtt-GFP and seeded with 0.5 mM KKQ40KK aggregates or vehicle control 24 hr later. Secreted ApiCCT1 (via the transfection of the secretible ApiCCT1 construct) blocked mHtt aggregation and/or seeding-enhanced aggregation process ($P < 0.0001$). Fluorescence microscopy-mediated quantification of aggregate-positive cells demonstrate that $<5\%$ of cells exhibit aggregates with or without oligomeric KKQ40KK seeding. In contrast, 64%-76% of cells with seeding and 32%-34% of cells without seeding manifesting intracellular aggregates in both controls (empty vector-transfected and un-transfected cells). Inhibition of mHtt aggregation is correlated with cell survival and corroborated by biochemical analysis.
Conclusions: Our studies indicate that exogenous ApiCCT1 inhibits the intracellular aggregation of mHtt, at least in part, by blocking exogenous seeding. These results have important translational and therapeutic implications suggesting that exogenous ApiCCT1 could be therapeutic even if does not enter cells. These results further suggest that exogenous delivery of apiCCT1 may be sufficient as a therapy, and thus, providing an easier therapeutic goal than intracellular expression or delivery of CCT1.
Keywords: CCT1 Huntington's disease huntingtin chaperonin
Disclosure: S. Potkin, **Part 1:** Bristol-Myers Squibb, Eisai, Inc., Eli Lilly, Forest Laboratories, Genentech, Janssen Pharmaceutical, Lundbeck, Merck, Novartis, Organon, Pfizer, Roche, Sunovion, Takeda Pharmaceutical, Vanda Pharmaceutical, **Part 2:** Lundbeck, Merck, Novartis, Sunovion, **Part 3:** Lundbeck, Merck, Novartis, Sunovion, **Part 4:** Amgen, Baxter, Bristol-Myers Squibb, Cephalon, Inc., Eli Lilly, Forest Laboratories, Genentech, Janssen Pharmaceutical, Merck, Novartis, Otsuka, Pfizer, Roche, Sunovion, Takeda Pharmaceutical, Vanda Pharmaceutical, NIAAA, NIBIB, NIH/NCRR, University of Southern California, UCSF, UCSD, Baylor College of Medicine; Z. Tan, Nothing to Disclose; E. Mitchell Sontag, Nothing to Disclose; W. Bunney, Nothing to Disclose; L. Thompson, Nothing to Disclose; C. Glabe, Nothing to Disclose.

M108. Chronic Glucocorticoid Induces Parkin 2 – Mediated Ubiquitin-Proteasome Activity and TrkB Degradation

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Background: Stress and glucocorticoid hormones, which are released into the circulation following stressful experiences, have been shown to contribute significantly to the manifestation of various psychiatric illnesses including Post-Traumatic Stress Disorder (PTSD) and depression. The brain-derived neurotrophic factor (BDNF) signaling through its receptor TrkB plays a critical role in stress-mediated changes in structural as well as functional plasticity in the prefrontal cortex. Deregulation of TrkB expression and signaling in the prefrontal cortex, and elevated glucocorticoid levels are linked to schizophrenia, PTSD and depression. However, the molecular mechanisms that regulate TrkB receptor internalization and turnover remain unknown. The aim of this study is to determine the role of ubiquitination in glucocorticoid-induced downregulation of TrkB. Ubiquitination is a post-translational modification of proteins orchestrated by a well-defined process involving a cascade of three enzymatic activities. Of these, the final step catalyzed by the enzyme E3 ubiquitin ligase, such as Parkin 2, determines the specificity of substrate ubiquitination. The specific hypothesisbehind the proposed research is that chronic glucocorticoid exposure induces Parkin 2 - dependent downregulation of TrkB in the prefrontal cortex.
Methods: Cortical neurons were isolated from E-16 CD-1 mice. siRNA transfection in primary neurons were performed by Nucleofector method (Amaxa) according to the manufacturer's instructions. Neurons at Days *In Vitro (*DIV) 5 were treated with vehicle (DMSO) or Corticosterone (Sigma; 0–10 uM) for 24 hours. Immunopreciptation was performed to determine TrkB ubiquitination and to understand the interaction between TrkB and Parkin. Protein levels of Parkin (50-kDa), TrkB (148-kDa), Ubiquitin, and actin (42- kDa) were analyzed via western blotting. mRNA levels of Parkin and TrkB were analyzed via Real Time PCR. Values were normalized to two house keeping genes (actin and GAPDH). The expression of 84 key genes involved in the proteasomal degradation pathway was examined via Mouse Ubiquitylation Pathway RT^2 Profiler PCR Array (SA Biosciences). Cellular localization of TrkB in primary cortical neurons (DIV 14) was examined via immunofluorescence method. BDNF protein levels in neuronal extracts were examined by ELISA. Statistical analysis between groups was performed by unpaired two-tailed Student's *t* test.
Results: We found that chronic corticosterone treatment down-regulates TrkB protein expression, but not TrkB mRNA in primary cortical neurons. Chronic corticosterone treatment induced TrkB ubiquitination in neurons. PCR array data showed significant increases in genes associated with ubiquitination pathway in neurons following corticosterone treatment. Further analysis indicated that corticosterone exposure results significant increases in Parkin 2 (an E3 ubiquitin ligase) protein and mRNA expression in neurons. We found that Parkin is the E3 ligase associated with TrkB in neurons. Moreover, Parkin knockdown with small interfering RNA showed higher TrkB protein levels in mouse primary cortical neurons. Chronic corticosterone had no effect on BDNF protein levels in neurons. Immunofluorescence analysis confirmed coclocalization of Parkin 2 and TrkB in neurons.
Conclusions: Chronic glucocorticoid exposure induced posttranscriptional down-regulation of the BDNF receptor TrkB in primary cortical neurons with a concurrent increase in proteasome activity. Thus, inhibition of the ubiquitin-proteasome-mediated TrkB degradation may be an important mechanism for preventing defective BDNF/TrkB signaling and may offer a new strategy for treating stress-related neuropsychiatric disorders.
Keywords: stress, BNDF, TrkB, ubiquitin, Parkin, depression, neurons
Disclosure: A. Pillai, Nothing to Disclose; C. Pandya, Nothing to Disclose; C. Jowers, Nothing to Disclose.

M109. GLYX-13, a NMDA Receptor Glycine Site Functional Partial Agonist, Exerts its Antidepressant Effects by Acting at a Novel NMDA Receptor Modulatory Site

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Background: Modulation of NMDA receptor (NMDAR) activity has emerged as a viable therapeutic approach for depression. GLYX-13 is a Glycine Site Functional Partial Agonist (GFPA) currently in Phase II clinical development for treatment-resistant depression. GLYX-13 has been previously shown to preferentially modulate NR2B-containing NMDARs, as GLYX-13-mediated facilitation of NMDAR current at rat Schaffer collateral-CA1 synapses is

completely inhibited by the NR2B antagonist ifenprodil. The present studies detail the *in vitro* characteristics of GLYX-13.
Methods: Functional glycine site agonist effects were measured using an [^{3}H]MK-801 potentiation assay. Rat cortical membrane extracts were preincubated for 15 minutes in the presence of a saturating concentration of glutamate and various concentrations of compounds acting at the glycine site as indicated in Results. [^{3}H]MK-801 was then added, incubated an additional 15 min (i.e., under nonequilibrium conditions), and bound and free radioligand separated by rapid filtration. Functional antagonist effects were determined using pA2 analysis in both i) the [^{3}H]MK-801 assay and ii) NMDAR current from CA1 pyramidal neurons in rat hippocampal slices. Direct interaction of GLYX-13 at the glycine site of the NMDAR was assessed using radioligand displacement of i) [^{3}H]glycine and ii) [^{3}H]L-689,560. Functional interaction at NR2B-containing NMDAR was assessed in the [^{3}H]MK-801 assay in the presence of i) 5,7 dichlorokyneurenic acid and ii) Ro 25-6981. GLYX-13 was also tested for its functional agonist activity at the NMDAR glutamate and the polyamine sites. Finally, functional agonist effects of GLYX-13 on Ca^{2+} flux were measured in HEK293 cells stably expressing recombinant human NR1/NR2B receptors.
Results: [^{3}H]MK-801 binding studies showed that GLYX-13 exhibits partial agonist activity at the glycine site (EC_{50} 64 nM; 26.1% activity relative to glycine). As partial agonists also demonstrate antagonist-like activity in the presence of full agonist, the affinity of GLYX-13 to NMDAR was measured using the pA2 response shift method for partial agonists. GLYX-13 exhibits similar potencies in assays assessing channel opening in the [^{3}H]MK-801 assay (Kp: 6.5 μM) and by direct NMDAR current measured in hippocampal slices (Kp; 1.3 μM). Radioligand competition studies showed that GLYX-13 has no effect on [^{3}H]glycine binding in purified rat cortical membranes, affecting neither the affinity of glycine for the NMDAR nor the rate of binding. Additionally, while glycine and D-cycloserine competed with the high affinity glycine site agonist [^{3}H]L-689,560, GLYX-13 was ineffective. In a dose-dependent fashion, the NR2B specific inhibitor Ro 25-6981 antagonized the effect of GLYX-13 using the [^{3}H]MK-801 potentiation assay. GLYX-13 was also inactive at NMDAR glutamate and polyamine sites. Under the conditions used in these studies, GLYX-13 did not influence the ability of glutamate or spermidine to modulate NMDAR channel opening. Extending these results to human receptors, we created a doxycycline inducible HEK cell line expressing the functional human NR1/NR2B receptor subtype. Using this platform, and corroborating the [^{3}H]MK-801 results, partial agonist activity of GLYX-13 at the glycine site was demonstrated (EC_{50}: 52 nM; 43.8% activity relative to glycine). Also corroborating the [^{3}H]MK-801 results, no activity at the glutamate site was measured.
Conclusions: Taken together, the present studies provide data that GLYX-13 is a glycine site functional partial agonist at NR2B-containing NMDARs. Because GLYX-13 does not directly interact at the glycine, glutamate, or polyamine sites, it likely acts at a novel modulatory site on the NMDAR.
Keywords: NMDA Receptor, Depression, GLYX-13
Disclosure: J. Moskal, **Part 1:** Naurex Inc.; R. Kroes, **Part 1:** Naurex Inc.; J. Burgdorf, **Part 1:** Naurex Inc.; A. Gross, **Part 1:** Naurex Inc.; X. Zhang, **Part 1:** Naurex Inc.; R. Burch, **Part 1:** Naurex Inc.; P. Stanton, **Part 1:** Naurex Inc.

M110. Next Generation Sequencing Using ChIP-Seq Highlights an Essential Role for SIRT1 in Emotional Plasticity

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Background: Chromatin immunoprecipation (ChIP) combined with high-throughput sequencing (ChIP-seq) has become the technique of choice for whole-genome mapping of protein-DNA interactions. In this study we evaluate the role of SIRT1 and its downstream targets as potential new candidates for the treatment of neuropsychiatric disorders by performing chromatin immunoprecipitation followed by genome-wide profiling (ChIP-seq) in nucleus accumbens (NAc) tissue from drug treated and socially defeated stressed mice. The most well studied sirtuin is SIRT1, which is widely known for its role in regulating the acetylation state of histones and non-histone proteins. This, in turn, influences gene expression and cellular physiology. SIRT1 can affect the acetylation of the four core histone proteins *in vitro*, but seems to preferentially deacetylate histone 3 on lysines 9 and 14 (H3K9 and H3K14) and histone 4 on lysine 16 (H4K16). SIRT1 is also known to mediate a wide range of physiological processes, including cell differentiation apoptosis, autophagy, development, cancer metabolism, and circadian rhythms.
Methods: In this study we utilized the technique of ChIP-seq, which provides several advantages over ChIP-chip, such as less starting material, lower cost compared with whole genome tiling array, full genome coverage and higher peak resolution. Briefly, for each ChIP-Seq, bilateral 14 gauge NAc punches were pooled from eight mice, the tissue was then lightly fixed to cross-link DNA with associated proteins. Oligonucleotide adapters were then added to the small stretches of DNA that were bound to the protein of interest to enable massively parallel sequencing. For the social defeat stress paradigm, briefly 5 min defeats were carried out by placing an experimental mouse in the home cage of a different aggressive CD1 mouse each day for 10 days. After each defeat, the mice were separated by a plastic barrier with holes to allow nonphysical aggressive interaction to continue for 24 hr. Social interaction with a novel mouse was measured 24 hr after the last defeat.
Results: First, we observed that chronic social defeat stress modulates induces SIRT1 levels in the NAc. To directly determine whether SIRT1 in the NAc is a causative factor in regulating baseline depression-like behaviors in the absence of stress, we increased SIRT1 levels by injecting HSV-SIRT1 into the NAc of wild-type mice. We found that increasing SIRT1 resulted in an increased in depressive- and anxiety-like phenotypes similar to that observed after chronic social defeat when measured on several measures of anxiety and despair. First, using an open field paradigm, we found that HSV-SIRT1 in the NAc increases anxiety-like behavior as measured by decreased in time spent exploring the center of an open field maze ($p < 0.05$). These effects were not associated with changes in other performance variables such as velocity, total distance traveled, and time in periphery. When tested on a second measure of anxiety, animals expressing HSV-SIRT1 mice displayed a decrease in time spent exploring the open arm of the elevated plus maze (EPM) ($p < 0.05$) . On a measure of despair, the forced swim test, an assay of acute stress responses often used as a screen for antidepressant activity, HSV-SIRT1 overexpression in NAc resulted in a robust increase in immobility time ($p < 0.05$), typically interpreted as a pro-depression-like effect.
Conclusions: The results suggest that SIRT1 plays an essential role in mediating anxiety and despair-like behaviors and represents a potential novel target for the development of future antidepressants.
Keywords: Epigenetics, Stress, DNA Sequencing, Chromatin, Depression
Disclosure: D. Ferguson, Nothing to Disclose; N. Shao, Nothing to Disclose; J. Koo, Nothing to Disclose; J. Feng, Nothing to Disclose; V. Vialou, Nothing to Disclose; R. Neve, Nothing to Disclose; L. Shen, Nothing to Disclose; E. Nestler, Nothing to Disclose.

M111. Glucocorticoid Receptor Translocation is Attenuated in Olfactory Neuroepithelial Cells of Patients with Major Depressive Disorder

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Background: Cellular responsivity to glucocorticoids (GC) plays a pivotal role in the complex interplay between stress and depression. Critical for GC responsivity is translocation of glucocorticoid receptors (GRs) into the nucleus, a process which is governed by chaperone complexes comprised of the immunophilins; FKBP51, FKBP52, the heat shock protein, hsp90, and the molecular chaperone protein, BAG1. Of import, some of these genes, FKBP5, FKBP4 and BAG1, have been implicated in the pathophysiology of depression and stress related disorders. Thus, GR chaperone complexes, protein interactions in particular, could be a point of convergence for various etiologic/risk factors for depression and stress responses. GC signaling in patients with MDD has been extensively studied but mostly in non-neuronal cells. The goal of this study was to examine GR signaling in neural cells derived from patients with MDD using the olfactory neuroepithelial biopsy. In addition, we have examined olfactory neuroepithelial biopsy tissues obtained from mice challenged with social defeat, which permits a bilateral translation with which to delineate altered GR function and chaperone complexes as new molecular substrates for the interactions between stress and depression.

Methods: OE biopsies were obtained from subjects ages 22-59, in collaboration with the department of Otorhinolaryngology in accordance with an IRB approved protocol at the University of Pennsylvania. Two tissues were obtained from each subject from the high middle turbinate, placed in culture to generate neuroepithelial (OE) cells. OE cells were derived from 15 age-sex matched pairs of subjects with MDD and healthy controls and were examined for GR translocation, protein expression, mRNA expression and protein association in the GR chaperone complex. Dexamethasone-induced GR translocation and was tested using two methods; the ImageXpress Micro imaging system and Western blot. Protein expression levels were examined with western blot and mRNA expression with qPCR. Protein associations of GR chaperone proteins were tested with immunoprecipitation experiments using hsp90 as the bait to capture complexes containing GR and FKBP51. To assess GR translocation by western blot, OE cultures were incubated with 1uM dexamethasone for 15 minutes and processed for nuclear and cytosolic fractions. For assessment of GR translocation by IX microscopy, OE cultures were plated in 96 well plates in triplicate, incubated with dexamethasone 1uM for 15 minutes and immunolabeled for GR in the nucleus versus cytosol.

Results: GR translocation, as measured by nuclear to cytosol ratio of GR on western blot before and after dexamethasone treatment, was strikingly reduced in OE culture cells from depressive patients compared to controls (paired t-test, two tailed, $p = 0.0002$, $t = 5.167$, $df = 12$). Interestingly, protein associations within chaperone complexes were found to be altered in the patient group. The association of GR with FKPB51, was significantly increased in subjects with MDD compared to their matched controls (paired t-test, two tailed, $p = 0.037$, $t = 2.568$, $df = 7$). GR-FKBP51 association is known to decrease GR translocation. Furthermore we found that FKBP51-GR association was inversely correlated with GR translocation ($p = 0.05$). These altered protein associations in chaperone complexes in MDD may not be a direct result from regulation of gene expression. mRNA levels of hsp-90, FKBP51 or FKBP52 didn't differ between the two groups. At the protein level, we found a trend for an increase in FKBP-51 and for a decrease in hsp-90 in the patient group. Interestingly, these trends for changes in FKBP-51 and hsp-90 were also found in mice exhibiting vulnerability to social defeat compared to resilient ones.

Conclusions: GC signaling has been extensively studied as a mechanism for GC resistance and/or hypercortisolemia in depression yet mostly in non-neuronal cells of MDD patients. This study presents the first direct evidence for decreased GR translocation in neural cells of patients and suggests that glucocorticoid resistance in depression may be partly due to decreased GR translocation via altered protein composition of the chaperone complexes. The majority of patients were in a current major depressive episode at the time of olfactory biopsy, thus limiting our ability to distinguish between state and trait in relation to the molecular changes in GR. Several subjects in the study were treated with antidepressants at the time of biopsy but we found no correlation between medication status and GR translocation. FKPB52 is known to sequester GR in the cytosol, thus the increased association of FKBP51 with GR may be at least in part responsible for the decreased GR translocation we observed in olfactory epithelial cells from MDD patients. It is unclear whether altered GR's association with its chaperones results from dysregulation of their expression. It is interesting, however, that we observe parallel changes in the expression of FKBPs and hsp-90 in depressed patients and stress vulnerable mice, further supporting the olfactory epithelial biopsy approach as a bridge for a bilateral translation. Together the results of our study suggest that GR translocation mediated by chaperone complexes could be molecular substrates that hold clues to complex interactions between stress responses, depression and their modulation.

Keywords: major depressive disorder glucocorticoid stress olfactory chaperone complex

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M112. PSD Protein Partitioning is Drastically Altered in the Lateral Prefrontal Cortex of Schizophrenia

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Background: Decreased synaptic connectivity is currently a leading postulate for the synaptic pathophysiology of schizophrenia. Critical to this postulate is histologic evidence that dendritic spine density is decreased in the DLPFC and other brain regions of schizophrenia patients. Dendritic spines harbor the post-synaptic density (PSD), a synaptic microdomain integral to neuroplasticity. Thus, decreased spine density and neuroplasticity impairments may reflect abnormalities in PSD function. Neuroplasticity is a complex process involving hundreds of proteins organized into nonlinear signaling pathways. Many of the most reproducibly identified SCZ risk genes impact neuroplasticity pathways at the PSD, suggesting that the PSD may serve as a point of convergence for genetic risk factors. Historically, neuroplasticity has been investigated with respect to a limited number of proteins; yet it has become increasingly clear that a full view of neuroplasticity

requires simultaneous assessment of multitudes of synaptic proteins. The goal of this project is to decipher the PSD protein constellation in comparison to protein expression in schizophrenia by employing newly developed microdomain specific proteomic methods.
Methods: We have developed a Liquid Chromatography-Selected Reaction Monitoring/Mass spectrometry (LC-SRM/MS) method for the highly sensitive, accurate (avg $R^2 = .96$) and precise (avg CV = 3.8%) quantification of over 250 selected synaptic proteins. This approach utilizes a [$_{13}C^6$] lysine-labeled brain proteome internal standard prepared from stable isotope labeled mouse brain tissue. This assay was first used to further validate biochemical fractionation of human postmortem brain tissue in whole tissue homogenates, PSD, vesicular, parasynaptic and synaptosomal preparations from the brain tissue of three normal human subjects and three mice. Next, whole tissue homogenates and PSD enrichments were prepared from the postmortem LPFC of 15 matched pairs of SCZ and control subjects and analyzed by LC-SRM/MS. To control for neuroleptic effects, an identical analysis was performed on cortex tissue from rhesus monkeys treated for 6 months with haloperidol, clozapine or vehicle (n = 10). Protein expression levels in whole tissue homogenates were calculated with β-tubulin as a normalization factor while protein levels in PSD fractions were normalized to PSD95. Protein enrichment ratios in PSD fractions were calculated by normalizing PSD protein values to whole tissue homogenate protein values. DAVID Functional Annotation Tool was used to search for enriched Kegg pathway and gene ontology terms in the list of regulated proteins using all assayed proteins, not the whole genome, as background. Significance of diagnosis or neuroleptics on expression or enrichment of protein groups identified by DAVID was assessed by ANOVA in GraphPad Prism 5. Unsupervised hierarchical clustering was used to construct self-organized heat maps of protein and subject by non-normalized PSD protein values in Cluster 3.0 and Treeview.
Results: Protein enrichments in subcellular fractions prepared from human postmortem brain tissue were strikingly similar to those prepared from fresh mouse brain tissues. Analysis of whole tissue homogenates showed no significant differences between the patient and control groups except two protein families; CaMKIIs ($p = .0002$) and proteolytic enzymes ($p = .0012$), which were significantly increased in the patient group. In PSD fractions prepared from the LPFC of SCZ and matched control subjects, a striking between-group difference was seen in the ratios of proteins amounts in the PSD with respect to the same molecules in whole tissue extracts, called the enrichment ratios henceforth. In PSD fractions prepared from SCZ subjects, enrichment ratios for glutamate receptors, scaffolding proteins and voltage dependent anion channels, grouped under the functional annotation *post-synaptic cell membrane* ($p = .0007$) were significantly increased (+40%, $p < .0001$). However, levels of these proteins in PSD fractions were mostly unaltered between the two groups when normalized to PSD-95. Unsupervised hierarchical clustering of non-normalized PSD protein values segregated control and SCZ subjects into two groups with near perfect accuracy. Contrasting the findings in human tissue, enrichment ratios for this same group of PSD proteins were *decreased* in PSD fractions prepared from rhesus monkeys chronically treated with haloperidol compared to vehicle ($p = .0076$).
Conclusions: Decreased spine density observed in schizophrenia may have predicted suboptimal development of the PSD and concordant decreases in PSD protein expression. The results of our study, however, failed to show such decreases either in whole tissue or in PSD fractions. Instead, the ratios of proteins in the PSD with respect to those in the whole tissue extracts were significantly increased specifically for the core PSD proteins in schizophrenia. We propose that alterations in partitioning of PSD proteins, not in expression, may be a molecular correlate of decreased spine density in schizophrenia. Given that PSD partitioning was found to be altered specifically in the core PSD proteins, it is tempting to speculate that our observations may reflect changes in ultra-structure of the PSD. This altered partitioning could result from unstable synapse formation/maintenance or aberrant trafficking events and should be observable by immunohistochemistry and/or electron microscopy.
Keywords: Postmortem, Schizophrenia, Neuroplasticity, Proteomics, Postsynaptic Density
Disclosure: M. MacDonald, Nothing to Disclose; G. Ciccimaro, **Part 2:** ThermoFisher Scientific; S. Siegel, **Part 3:** Consultant for NuPathe, Abbott and Lundbeck, **Part 4:** Astellas; S. Hemby, **Part 4:** Astra Zeneca; I. Blair, **Part 4:** Johnson and Johnson; C. Hahn, **Part 4:** Pfizer Phramaceutical

M113. Developmental Switch in Striatal Gene Expression in Rat: Implications for Schizophrenia

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Background: Brain defects during development are significant risk factors for schizophrenia. Insults during the second trimester, a period of active striatal development, are associated with increased risk for schizophrenia. The equivalent stage in rats occurs during the second postnatal week. Because abnormal development of striatal medium spiny neurons may lead to pathology underlying major psychiatric illnesses, we studied the expression pattern of genes involved in striatal development and genes associated with key MSN-specific pathways during the first two postnatal weeks in rat.
Methods: Using subtractive hybridization, we have identified over 60 genes, many not previously known to play a role in neuromaturation. The developmental expression of these genes was determined by quantitative real-time PCR.
Results: We show that during the first two postnatal weeks in rat, an early gene expression network, lacking key MSN-specific signaling pathways, is downregulated and replaced by a mature gene expression network, containing key MSN-specific genes, including the D1 and D2 receptors, conferring to these neurons their MSN functional identity. We have also identified 12 novel transcripts, which do not match known genes, but which show strict developmental expression and likely play a role in striatal neurodevelopment.
Conclusions: Striatal neurons undergo a strictly timed developmental switch in gene expression networks, which contain key striatal projection neuron genes, such as the D1 and D2 receptors. Therefore, before this developmental switch, striatal neurons lack their MSN phenotype. We show that this maturation process is followed by a striking striatal-specific myelination event. As many strictly controlled developmental processes, it is likely to be susceptible to environmental insults. Indeed, this period is known to be a susceptibility period in both humans and rats.
Keywords: striatum, developmental expression, schizophrenia, dopamine receptor, neuromaturation
Disclosure: G. Novak, Nothing to Disclose; T. Fan, Nothing to Disclose; B. O'Dowd, Nothing to Disclose; S. George, Nothing to Disclose.

M114. A Potential Mechanism of Behavioral Alteration by Genome Diversification: The Role of Neural MILI/piRNA Complexes on *De Novo* L1 Retrotransposition

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Background: Mobile retrotransposable elements, such as the long interspersed element (LINE) L1, can insert copies of themselves

throughout the genome and influence the expression of nearby genes. This is one of the mediators of genome diversification. Recent findings show that L1 element insertion can occur in dividing neurons, resulting in somatic cell diversification and possibly neuropsychiatric disorders such as Rett syndrome and schizophrenia. Our group is interested in developmental pathways leading to neuropsychiatric disorders. As such, while examining mechanisms of paternal influence on offspring behavioral outcomes, we discovered a possible role of the MILI/piRNA system as mechanism of neural L1 suppression. The mouse MILI gene is a member of the well-conserved PIWI family of proteins that are expressed across phylogenetically diverse species. Canonically, the MILI/piRNA complex has been shown to suppress L1 activity in the male germline, but a role for regulating L1 expression in dividing neurons has not been established. We hypothesized that there is a MILI/piRNA system in the brain and it has a direct impact on the behavior of the mouse by the regulation of L1 activity.

Methods: The expression of MILI in the mouse brain was detected by: real-time PCR, western blot hybridization and *in situ* hybridization (data taken from Allen Brain Atlas). Brain samples from wildtype (WT) and MILI$^{+/-}$ mice of C57BL/6 background were used to compare MILI expression change between the two sets of samples. In order to understand the impact of neural MILI on neuronal L1 expression, semi-quantitative reverse transcription PCR probing the expression of a retrotransposition competent L1 transcript from the L1 A subfamily was performed. To examine whether there are changes in the behavior of these two sets of mice, our breeding approach was to mate C57BL/6 MILI+/+ and +/− sires (but not −/− sires, since they are sterile) with WT C57BL/6 females. We then examined several different behavioral dimensions using an array of behavioral tests such as: the open field, light-dark choice test, social interaction (SI), elevated plus maze and forced swim test (FST).

Results: Contrary to what is generally thought to be a male mouse germline specific gene, here we show that, MILI, is also expressed in the mouse brain. Both MILI mRNA and protein expression are significantly reduced in the brains of MILI$^{+/-}$ compared to MILI$^{+/+}$ mice. We further observed that L1 expression is significantly increased in the brains of MILI$^{+/-}$ offspring compared to MILI$^{+/+}$ offspring of the same fathers. We observed that MILI$^{+/-}$ offspring had depression and SI related behavioral changes compared to MILI$^{+/+}$ offspring (demonstrated by increased floating duration during FST and increased time spent with social target during SI test, respectively). It could be argued that this change in MILI$^{+/-}$ offspring is due to inheritance of genetic diversity that arose in the germline of MILI$^{+/-}$ fathers, in this case we would observe changes in all offspring of MILI$^{+/-}$ fathers compared to offspring of MILI$^{+/+}$ fathers. However, changes in behavior were only observed in MILI$^{+/-}$ offspring, but not MILI$^{+/+}$ offspring of the same MILI$^{+/-}$ fathers. Based on the data, we now know that changes in the behavior of MILI$^{+/-}$ offspring is due to a direct effect of the MILI gene and independent of paternal germline MILI expression.

Conclusions: In the course of examining the effect of reduced paternal MILI expression on offspring behavior, we unexpectedly observed an independent effect of inheriting a null MILI allele on offspring behavior. This observation surprised us because MILI activity (if indeed testes specific) should not affect the brain. Here, we show that MILI is expressed in the brain. We wondered whether the MILI/piRNA complex might also function in dividing neurons. If so, the MILI haploinsufficient mice might exhibit behavioral differences due to an increase in *de novo* L1 insertions. Indeed, we observed increased L1 expression in the brains of MILI$^{+/-}$ mice. Together, these observations will allow future work to directly assess the presence of piRNA species in neurons and determine whether MILI/piRNA complexes control *de novo* L1 insertion in neurons. Somatic L1 insertions in neural populations has been proposed as a mechanism for generating behavioral diversity as well as a potential etiology for neuropsychiatric disorders. A better understanding of the role of the MILI/piRNA system in L1-mediated neuronal genome diversification may lead to new insights into the origins of certain neuropsychiatric disorders as well as new preventative strategies.

Keywords: MILI/piRNA complexes, L1 retrotransposon, genome diversification, behavior alteration, neuropsychiatric disorders
Disclosure: D. Lin, Nothing to Disclose; J. Gingrich, Nothing to Disclose.

M115. Top-down Control of Raphe Circuits in Affective Resilience: Key Role of Raphe GABA Interneurons

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Background: Imaging studies have revealed dysfunctions in the ventromedial prefrontal cortex (vmPFC) of patients with major depressive disorder (MDD). Deep brain stimulation (DBS) targeting the vmPFC is therapeutic in patients suffering from treatment resistant depression and produces antidepressant-like responses in rodents. Lesion studies in rodents suggest that serotonin (5-HT) partly mediates this antidepressant-like therapeutic activity of vmPFC DBS. The top-down control exerted by vmPFC over raphe circuits has also been implicated in stress coping and the expression of affective resilience. Underlying circuit mechanisms are poorly understood. The median and dorsal Raphe Nuclei (RN) receive strong excitatory inputs from the vmPFC. Although the RN contains a large proportion of 5-HT neurons, previous reports suggest that excitatory mPFC inputs preferentially synapse onto GABAergic interneurons. Given their position as primary postsynaptic target for vmPFC inputs in the RN, we hypothesized that Raphe GABA interneurons may be pivot to regulate vmPFC-RN connectivity during stress and the expression of affective resilience

Methods: We used combined genetic, electrophysiological and behavioral approaches to dissect the function of RN GABA interneurons in the social defeat (SD) mouse model of depression. Reporter mice with fluorescently-tagged GABA (GAD-Tomato) or 5-HT neurons (Pet1-tomato) were exposed to 10 days of social defeat and were segregated into resilient and vulnerable subpopulations based on social avoidance tests. SD-induced cFos was mapped using immunohistochemistry. Whole cell recordings of genetically identified neurons were conducted to characterize SD-induced changes in intrinsic properties of RN neurons and their synaptic inputs. mPFC-RN connectivity was assessed morphologically using viral-mediated track tracing and functionally using optogenetic stimulation and cFos mapping. To examine the role of RN excitatory inputs, ChR2 was targeted to vmPFC pyramidal neurons using an AAV vector under the control CamK2a promoter. Axon terminals of transduced neurons were photostimulated locally in the DR. To examine the role of local 5-HT and GABA neurons in the expression of vulnerable and resilient behavioral phenotypes, the proton pump Arch was targeted to GABA or 5-HT neurons thereby allowing selective photosilencing of each cell populations. Photostimulation and photosilencing were conducted in freely moving mice, either during the sensory phase of social defeat training or during social interaction tests.

Results: We found GABA neurons to be preferentially activated over 5HT neurons after repeated experiences of SD. cFos induction by SD was significantly greater in stress-resilient than vulnerable mice and was topographically distributed in the RN. A strikingly similar pattern of activation was observed following direct photostimulation of vmPFC terminals in the RN. Using AAV-mediated anterograde tracing we observed a topographical overlap between the distribution of cFos expressing GABA neurons in the

RN and the distribution of mPFC terminals, a result suggesting that vmPFC inputs drive GABAergic activation in RN during defeat stress and the degrees of this mPFC-driven GABAergic activation predicts subsequent social avoidance. In contrast to cFOS data, whole-cell electrophysiology of genetically identified GABA neurons revealed a diminished glutamatergic input in resilient mice. These results suggest that repeated phasic activation of RN GABA neurons during SD in resilients may trigger neuroadaptations that result in a tonic reduction of glutamatergic synaptic inputs. Lastly, we found that photosilencing DRN GABA neurons during the sensory contact period that follows physical defeat but not social interaction test, promoted the expression of a resilient phenotype and fully prevented social avoidance.
Conclusions: These results highlight a key role for DRN GABAergic neurons in expression of resilience to social defeat and stress induced neuroplasticity of mPFC raphe circuits.
Keywords: Social Defeat, Raphe Nuclei, vmPFC, resilience, Optogenetics, depression
Disclosure: C. Challis, Nothing to Disclose; J. Espallergues, Nothing to Disclose; S. Beck, Nothing to Disclose; O. Berton, Nothing to Disclose.

M116. Ras Suppressor 1 Acts Downstream of Integrin to Regulate Rac1 Activity and Ethanol Consumption in Drosophila and Humans

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Background: Alcohol abuse is a health threat that affects millions of individuals. A major factor underlying the development of alcoholism is the genetic makeup of an individual, yet much remains to be understood about the genes and signaling pathways underlying the development of alcoholism.
Methods: Mutant fly strains were isolated in a forward genetic screen, using a transposable element as a mutagen. Flies were exposed to vaporized ethanol to determine alcohol-induced sedation, and the capillary feeder (CAFÉ) assay was used to measure ethanol consumption preference.
Results: We show that mutations in the Drosophila *ics* gene, encoding the Ras suppressor 1 (Rsu1) protein, cause resistance to ethanol-induced sedation. Function of Rsu1 is required in the adult nervous system for normal ethanol-induced behavior. We show that Rsu1 acts downstream of the integrin cell adhesion molecule, and upstream of the actin regulatory GTPase Rac1. We also find that Rsu1 directly binds to Rac1, to regulate its activity. To test if Rsu1 is required for ethanol preference, we tested Rsu1 mutants in a two-bottle choice paradigm. Our results indicate that flies lacking Rsu1 show increased preference for ethanol. We also show preliminary data, that human *Rsu1* variants are associated with high alcohol consumption.
Conclusions: Our data show shat Rsu1 is required in flies for proper ethanol-induced sedation, and alcohol consumption preference. Since human *Rsu1* variants are also associated with increased alcohol consumption, our data highlight the translational value of Drosophila genetics, and how flies can contribute to the mechanistic understanding of pathways regulating alcohol abuse disorders.
Keywords: alcohol, addiction, genetics, Drosophila, human
Disclosure: S. Ojelade, Nothing to Disclose; G. Schumann, Nothing to Disclose; A. Rothenfluh, Nothing to Disclose.

M117. Cellular Mechanisms of Growth Arrest and DNA Damage-inducible 45b (Gadd45b) in Regulating Antidepressant-induced Adult Hippocampal Neurogenesis

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Background: Major depression, a common mental illness affecting millions of people worldwide, is one of leading causes of morbidity and has a significant economic cost. All common used classes of antidepressants and electroconvulsive therapy (ECT) promote proliferation of neural progenitors in the adult dentate gyrus, which in turn has been suggested to be important for antidepressant-induced behavioral effects. Previously, we showed that Growth arrest and DNA damage-inducible 45b (Gadd45b) is essential for ECT-induced, but not basal, levels of neural progenitor proliferation and dendritic growth in newborn neurons of the adult brain. However the cellular target of Gadd45b in regulating one or more of the steps of neural progenitors in response to ECT has not been fully established. Here we identified Gadd45b serves as a key molecular factor in ECT-induced of neurogenesis of the adult brain by modulating radial glial-like neural stem cells. These findings suggest a cellular mechanism of Gadd45b in regulating ECT-induced adult neurogenesis through modulating radial glial-like neural stem cells.
Methods: In order to examine the cellular target of Gadd45b in regulating neural progenitors, we utilized multiple histological techniques, confocal imaging, retroviral mediated approach and animal genetic model systems.
Results: During adult hippocampal neurogenesis, activation of quiescent radial glial-like neural stem cells (RGL) prodcues intermediate progenitor cells (IPCs), which in turn give rise to newborn dentate granule neurons. We demonstrated that deletion of Gadd45b regulates several essential steps of adult hippocampal neurogenesis *in vivo*, including proliferation of neural progenitors and dendritic growth of newborn dentate granule cells in the adult brain in response to ECT. Further, our quantitative analysis also showed that ECT markedly enhanced the overall number of RGL and IPCs in WT mice. This ECT-induced number of RGL and IPCs was significantly attenuated in KO mice, whereas the basal level of RGL and IPCs was similar. Thus, Gadd45b is essential for ECT-induced activation of RGL in adult dentate gyrus.
Conclusions: Our study demonstrates cellular target of Gadd45b in regulating adult neurogenesis in response to antidepressant and suggests a potential therapeutic target for the treatment of depression. These discoveries provide a basic foundation for understanding mechanisms of antidepressant action and thus may lead to a novel perspective for the development of therapeutic interventions.
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Keywords: Electroconvulsive therapy, Gadd45b, Adult neurogenesis, Radial glial-like neural stem cells
Disclosure: M. Jang, Nothing to Disclose; H. Jun, Nothing to Disclose; G. Ming, Nothing to Disclose; H. Song, Nothing to Disclose.

M118. Neuronal-Glial Interactions in the Nucleus Accumbens Following Morphine Administration: A Role in Relapase Behavior.

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Background: Glia, including astrocytes and microglia, have the ability to profoundly modulate neuronal function and behavior; however, very little is known about the signaling molecules that govern neuron-glia communication and in turn affect behavior.

We have recently determined that morphine treatment activates microglia in the nucleus accumbens (NAcc) to induce the local synthesis of cytokines and chemokines. This has important implications for addictive behavior as blocking morphine-induced glial activation using the non-specific glial inhibitor, ibudilast, has no effect on the initial rewarding properties of morphine, but completely prevents the relapse of drug-seeking behavior months later. Thus, we sought to determine 1) the glial source of these cytokines and chemokines in the NAcc in response to morphine, and 2) in what capacity neurons respond to these signaling molecules being released from glia.
Methods: To do this, we used fluorescence-activated cell sorting (FACS) of live neurons (Thy1 +), astrocytes (GLT1 +), or microglia (CD11b +) from the NAcc for the analysis of cell type specific gene expression following morphine or saline treatment.
Results: We determined that microglia are the primary producers of chemokine ligand (CCL) 4, CCL17, and their specific receptor CCR4; and morphine significantly increases the production of these chemokines and their receptor in microglia. CCL25 and its receptor, CCR9, are produced by microglia and neurons; however, only neurons respond to morphine treatment with a significant increase in CCL25. Neurons are the only cell type analyzed that produces the chemokine, CX3CL1, while microglia are the only cell type that bear its receptor, CX3CR1. Morphine treatment down-regulates the production of CX3CL1 from neurons, which has been shown to increase the activation of microglia. Microglia are the only cell type that express toll-like receptor (TLR) 4, a receptor to which morphine can bind directly. Interestingly, while the dopamine receptors D1r and D2r are primarily expressed by NAcc neurons, microglia also express significant levels of these same receptors.
Conclusions: These data indicate that morphine elicits a significant chemokine response from microglia either via direct activation of TLR4 and/or dopamine receptors, or indirectly via the neuronal down-regulation of CX3CL1 or the neuronal up-regulation of CCL25. In turn, neurons also have the capacity to respond to CCL25 produced by morphine treatment. Future studies will examine the mechanism(s) by which neurons respond to these immune signals produced by microglia in an effort to understand their profound effect on addictive behaviors.
Keywords: microglia, astrocytes, neurons, morphine, FACS
Disclosure: J. Schwarz, Nothing to Disclose; S. Bilbo, Nothing to Disclose.

M119. Limited Contribution of NMDA Receptor GluN1 Deletion in Cortical Excitatory Neurons to Schizophrenia-like Phenotypes
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Background: Pharmacological and genetic studies support a role for NMDA receptor (NMDAR) hypofunction in the etiology of schizophrenia. While we have previously demonstrated that NMDAR obligatory subunit 1 (GluN1) deletion specific to corticolimbic interneurons in early postnatal development is sufficient to confer schizophrenia-like phenotypes in mice, the consequence of NMDAR hypofunction in cortical excitatory neurons is not well-characterized.
Methods: We utilized a novel G35-3-Cre/fGluN1 conditional knockout mouse line in which NMDAR deletion is confined to cortical excitatory neurons with postnatal onset, to investigate how NMDAR hypofunction in cortical excitatory neurons may contribute to schizophrenia-like phenotypes in adult mice. We subjected the animals to histological, electrophysiological and behavioral assays.
Results: We first confirmed by NMDA current measurement that postnatal GluN1 deletion is functionally confined to the cortex. CtxGluN1KO mice are also normal for hippocampus- and amygdala-dependent behavioral tasks. Next, we subjected the mutant strain to a behavioral battery for schizophrenia-like and other psychiatric disorder-like phenotypes. While the mutant mice were impaired in prepulse inhibition of the auditory startle reflex, mutant mice did not exhibit additional hallmark schizophrenia-like phenotypes spatial working memory, social behavior, saccharine preference, novelty and amphetamine-induced hyperlocomotion, and anxiety-related behavior were all unimpaired. Furthermore, no genotypic difference was observed in MK-801-induced locomotor activity, suggesting that the psychotomimetic action of MK-801 is not mediated by NMDAR hypofunction in cortical excitatory neurons. Finally, mutants showed negligible levels of reactive oxygen species (ROS) production following chronic social isolation, and recording of spontaneous IPSC events from mPFC excitatory neurons suggested no alteration in GABAergic activity.
Conclusions: Cortical excitatory neuron-restricted GluN1 KO mutant mice were impaired in attention-related behavioral tasks in the absence of additional behavioral or cellular phenotypes reflecting schizophrenia pathophysiology. Thus, NMDAR hypofunction in cortical excitatory neurons may have limited application as a genetic model for schizophrenia pathophysiology.
Keywords: glutamatergic neuron, NMDA receptor hypofunction, prepulse inhibition, schizophrenia, transgenic mice.
Disclosure: G. Rompala, Nothing to Disclose; V. Zsiros, Nothing to Disclose; S. Zhang, Nothing to Disclose; S. Kolata, Nothing to Disclose; K. Nakazawa, Nothing to Disclose.

M120. Comparing Genome-wide Association Results for Conditioned Fear in Two Advanced Intercross Mouse Lines: Implications for the Genetic Mapping of Complex Psychiatric Traits
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Background: Anxiety disorders are debilitating illnesses characterized by exaggerations of our normal and adaptive reaction to fearful or stressful events. The importance of genetic influences on anxiety disorders is well known, yet identifying the underlying genetic mechanisms has proven difficult. Translational mouse models can provide a powerful strategy for understanding the genetic and biological underpinnings of the acquisition of fear, as well as the etiologic processes related to anxiety. While the full spectrum of any human psychiatric disorder can never be fully recapitulated in a single mouse model, conditioned fear (FC) may provide a useful model for some components of anxiety disorders. FC is a form of Pavlovian learning and has long been implicated in the pathogenesis of anxiety disorders, is heritable in mice and humans, and has well-established neurological underpinnings. Genetic mapping in mice studies have traditionally used recombinant inbred lines (RI), backcrosses (BC), F_2 intercrosses or similar populations to identify quantitative trait loci (QTLs). These approaches have seldom allowed the causal genes or polymorphisms to be identified. That is because these populations have high statistical power for detecting genetic loci, but poor mapping resolution due to limited recombination. More recently, QTL mapping has been performed using highly recombinant populations such as advanced intercross lines (AILs) and outbred mice. Unlike traditional mapping populations, AILs and outbred mice show a rapid breakdown of linkage disequilibrium that allows for increasingly high resolution mapping.
Methods: Here, we used two genetically distinct AIL populations (an integrated analysis of 625 F_2 and 567 F_8 AILs derived from C57BL/6J x DBA/2J mice, along with 490 F_2 and 687 F_{34} AILS derived from LG/J x SM/J mice) to fine-map QTL associated with

FC. We measured FC using a three-day paradigm. First, mice were conditioned to associate a test chamber (context) and a tone (cue) with a 0.5 mA foot-shock on day 1. On day 2, mice were re-exposed to the context, and freezing behavior (a species specific measure of fear) was recorded. On day 3 mice were exposed to the tone in a different environment and freezing was measured to establish the level of learned fear of the tone. All experiments were approved by the University of Chicago IACUC and conducted in accordance with NIH guidelines.

Results: We conducted an integrated genome-wide association analysis in QTLRel and identified four highly significant QTL affecting freezing to cue in the C57BL/6J x DBA/2J AIL (on Chr 1, 2, 5 & 13), and six highly significant QTL associated with freezing to cue in the LG/J x SM/J AIL (on Chr 2, 4, 8, 10, 11 & 17). The average percent decrease in the 1.5-LOD support interval between the F_2 and the F_8 integrated analysis was 59.2%, as compared to an 88.6% reduction between the F_2 and the F_{34} integrated analysis. Interestingly, none of the QTLs between the two AILs overlapped. We assume this is because different alleles are segregating between the two populations and because we had incomplete power. Epistatic interactions could also be a factor in the lack of overlap. We have also exploited bioinformatic sequence data available for all founder strains to identify candidate genes based on the existence of non-synonymous coding polymorphisms. In addition, we utilized expression data from the C57BL/6J and DBA/2J founder strains to identify candidate genes based on the existence of gene expression data in amygdala, hippocampus, and whole brain.

Conclusions: We identified multiple candidate genes that may be relevant to fear learning in animal models (*Bcl2, Btg2, Dbi, Gabr1b, Lypd1, Pam and Rgs14*) or PTSD in humans (*Gabra2, Oprm1* and *Trkb*). These results will be compared to preliminary GWAS data obtained from outbred mice (CFW, n = 480), which we expect will possess even greater amounts of recombination than AILs. Our results demonstrate that the integration of F2 and AIL data maintains the advantages of studying endophenotypes for complex psychiatric traits in model organisms while significantly improving resolution over previous approaches.

Keywords: advanced intercross lines; conditioned fear; genome-wide association; posttraumatic stress disorder

Disclosure: C. Parker, Nothing to Disclose; G. Sokoloff, Nothing to Disclose; R. Cheng, Nothing to Disclose; S. Gopalakrishnan, Nothing to Disclose; N. Gonzales, Nothing to Disclose; J. Davis, Nothing to Disclose; A. Palmer, Nothing to Disclose.

M121. Genomic Sequencing and Linkage Analysis in Selectively Bred Rat Lines Identify Grm2 and Lcn2 Stop Codons as Functional Alleles Influencing Alcohol Preference

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Background: Efforts to identify genetic loci influencing complex disorders, such as alcoholism, have been hampered by the heterogeneity of genes driving the heritability. Selectively bred animal lines represent a potentially invaluable resource for locus identification in complex traits because selection collects many of the genetic variants influencing the trait and reduces this heterogeneity. Alcohol-preferring (P) and non-preferring (NP) rats, created from Wistar rats by 30–70 generations of selection, model human alcoholism. Quantitative trait loci (QTLs) for alcohol preference and consumption have been identified. However, the identities of functional variants as well as the genes in which they reside, remain elusive.

Methods: Exome sequencing was carried out to examine genetic differences in gene-coding regions between P and NP rats and identify genetic variants influencing alcohol preference. Effect of the variants on alcohol consumption and preference were confirmed by QTL analysis in F2 rats derived from intercross between inbred P and NP rats. Global gene expression differences between P and NP rats were profiled in the hippocampal transcriptome with RNA-Seq.

Results: From 6 P and 6 NP rats sequenced, we identified a total of 25,715 SNPs that showed a clear pattern of homozygous allelic segregation. A genomic map highlighting genome-wide allelic segregation between the two rat lines was constructed. Among the homozygously segregating SNPs, two stop codons and 36 missense variants predicted to affect protein functions were identified. P rats were homozygous for a stop codon variant of the metabotropic glutamate receptor 2 gene (*Grm2* *407). The loss of the receptor protein (mGluR2) was complete. QTL analysis revealed that the loss of mGluR2 resulted in 32% increase in alcohol consumption and 28% increase in alcohol preference in F2 animals. The *Grm2* *407 allele was also identified in Wistar rats (allele frequency: 0.086). P rats were also homozygous for a stop codon in the lipocalin 2 gene (*Lcn2* *137). *Lcn2* *137 was also linked to increased alcohol consumption and preference in F2 rats. Differences in hippocampal gene expression between the two rat lines were consistent with homozygosity of the *Grm2* stop codon in P rats. There was a significant over-representation of the differentially expressed genes (FDR < 0.05) involved in glutamate transmission and synaptic functions. Association of genetic variation of GRM2 with trait anxiety (harm avoidance) was observed in two independent human population samples although no association to alcoholism was found.

Conclusions: The complete segregation of *Grm2* *407 and *Lcn2* *137 in P and NP rats, and linkage of *the* two stop codon variants to alcohol preference and consumption, point to selection of *Grm2* *407 and *Lcn2* *137 for alcohol preference. Loss of function of mGluR2 protein in P rats was confirmed. Perturbation in expression of genes involved glutamate transmission and synaptic function in P rats may be in part or entirely attributable to the *Grm2* stop codon, which is homozygous in these animals and also abundant in outbred Wistar rats. In model systems such as the selectively bred P and NP alcohol preference model, genomic sequencing in combination with linkage analysis to eliminate alleles fixed by genetic drift enables the identification of functional alleles that influence the complex trait and are otherwise rarer in populations.

Keywords: Grm2, Functional variants, Alcohol preference, PNP rats, Exome sequencing

Disclosure: Z. Zhou, Nothing to Disclose; T. Liang, Nothing to Disclose; M. Kimura, Nothing to Disclose; Q. Yuan, Nothing to Disclose; M. Enoch, Nothing to Disclose; C. Hodgkinson, Nothing to Disclose; F. Ducci, Nothing to Disclose; M. Järvelin, Nothing to Disclose; A. Pouta, Nothing to Disclose; J. Tapocik, Nothing to Disclose; E. Barbier, Nothing to Disclose; M. Heilig, Nothing to Disclose; H. Edenberg, Nothing to Disclose; D. Goldman, Nothing to Disclose.

M122. Comparative Analysis of Differential Allele Gene Expression in the Mouse Brain

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Background: Common inbred mouse strains display significant differences in behavior, drug preference, and their responses to different pharmacological agents including alcohol. These strain differences are generally stable and therefore are likely to arise due

to the genetic difference between these inbred strains. Recently the genomes of 17 common strains of laboratory mice have been sequenced by the Sanger Institute. Comparison of these genome sequences revealed 4,458,004 and 4,468,071 SNPs that distinguish C57Bl6/J from 129S1/SvlmJ, and DBA/2J respectively. Comparison of gene expression between C57BL/6J and DBA2/J showed 1,727 genes to have significant expression differences. However, it has not been studied well that how the strain specific variants play a role to affect strain differences in behavior and gene regulations. In F1 mice derived from crossing two differential strains it is possible to identify genes that are differentially expressed between the two strains using the parental allelic information of the cSNPs and to ultimately identify the variations that give rise to the differential gene expression.
Methods: Strand-specific mouse forebrain mRNA transcriptome libraries were prepared from forebrains of male F1 hybrid mice derived from C57BL/6J x 129S1/SvlmJ crosses (and from the reciprocal cross 129S1/SvlmJ x C57BL/6J) and sequenced on an Illumina GAIIx sequencer. Strain-specific cSNPs identified by RNA-Seq were confirmed by exome sequencing the two individual parental strains using the GAIIx sequencer and Agilent SureSelect baits targeting all mouse exons. Strain specific cSNPs allowed the identification of genes that showed different levels of expression from the two parental chromosomes, either due to parentally derived imprinting or where the two chromosomes are transcribed at different levels. Sequenced reads were mapped to mouse reference sequences (UCSC mm9) and SNPs were identified using Illumina's CASAVA (version 1.8.1) with variantsNoCovCutoff option. The base counts were parsed for SNPs with $>=4$x coverage or SNP Q-value $>=20$. SNPs' genetic locations were identified based on RefSeq Genes from UCSC Mouse Genome mm9. SNPs near splicing sites in introns from RNAseq reads were excluded. Information of known mouse SNPs from dbSNP Build 128 was added if there was a match. Relative expression levels of the two parental alleles were determined by comparison of the number of counts for each allele at a given locus. RNA-Seq results were then filtered according to the FDR adjusted p-value (<0.05), SNP calling from Exome sequencing results to avoid reference biased analysis, and set cut-off number of sequence reads between 8 and 3000 to avoid results with non-specificity. Imprinted SNPs was assessed by requiring paternal or maternal bias greater than 50% in both cross. Strain specific genes were required by expression pattern in the same way and strain specific bias greater than two fold in both cross.
Results: We identified 1533 SNPs in 879 genes in the 129BL6F1 cross and 1335 SNPs in 898 genes in the reciprocal BL6129F1 cross. The disparity in the number of SNPs and genes between the two crosses arises due to the filtering of FDR p-value and SNP calling from Exom. Imprinting in previously known genes was confirmed for *Inpp5f*, *Sgce*, *Peg10*, *Ragrf1*, *H13*, *Impact*, and *USP29* where only the maternal or paternal allele was expressed reciprocally in the two crosses. In addition we identified 32 novel imprinted genes; 6 paternally expressed genes (*Fam160b1*, *Kdm4a Hyi*, *Fkrp*, *Usp38*, and *Osbpl10*) and 8 maternally expressed genes (*Rnmtl1*, *Rims2*, *4930572105Rik*, *Gm1337*, *Ttbk2*, *Mpdz*, *Cttnbp2*, and *2310008H09Rik*). We also identified 255 genes that showed consistent patterns of differences between expression levels of the C57BL/6J or 129S1/SvlmJ alleles in both the B6129SF1 and 129B6SF1 mice. For 180 of these genes the c57BL/6J allele was consistently expressed at higher levels compared to the 129S1/SvlmJ allele and for 75 genes this was reversed.
Conclusions: Many of the differences observed between inbred mouse strains arise due the complex interplay of multiple genes and gene products. Although there are many missense variants between theses strains that alter the coded amino-acids in the protein products and which could alter function, some of the differences may arise due to differences in levels of expression of some genes. Using inbred strains which are homozygous through the majority of the genome it is possible to easily identify strain specific alleles and to use these alleles as surrogates to identify regulatory loci that modulate this differential regulation of gene expression. Next-generation sequencing has allowed the analysis of strain-specific differential expression on a genomic scale. Using this approach we have identified 32 additional genes that are subject to parental imprinting effects, none of which had previously been identified to be regulated in this manner. Identification of 255 genes that are consistently differentially regulated in these F1 mice is the first step that will allow us to identify the regulatory elements and genomic variants that give rise to observable strain differences at the phenotypic level.
Keywords: Allele specific expression, imprinting
Disclosure: S. Yeo, Nothing to Disclose; Z. Zhou, Nothing to Disclose; C. Hodgkinson, Nothing to Disclose; Q. Yuan, Nothing to Disclose; J. Jung, Nothing to Disclose; M. Enoch, Nothing to Disclose; D. Goldman, Nothing to Disclose.

M123. Open Field Testing of Drosophila Adults to Study CNS Stimulants

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Background: The fruit fly, *Drosophila melanogaster* is a powerful genetic model system. It exhibits many sophisticated behaviors including aggression, circadian, learning and memory, and courtship, each mediated by neurotransmitters and systems conserved with higher order animals like humans. These include dopamine, serotonin, glutamate, and GABA, among many others. The fly brain itself is composed of over 100,000 neurons arranged into distinct neuropil with specialized regions, similar to mammalian brain. One fruitful area the fly has been used for as a discovery platform is the study of drugs of abuse like cocaine and ethanol. As in humans, the effects of abused drugs like cocaine and ethanol in the fly depend on the neurotransmitter dopamine. High throughput screens have identified many genes that underlie the effects of these drugs, and genes and molecular mechanisms discovered in the fly have been translated back to mammalian systems to inform on key aspects of mammalian drug response. Most assays to date investigating CNS stimulants have simply examined activity scores. We have recently developed open-field testing for CNS stimulants to use in conjunction with activity monitoring. Together, these assays will be more powerful in elucidating molecular and genetic mechanisms underlying drug response, and enhance fundamental knowledge to translate back to mammalian systems.
Methods: Flies were fed drug either acutely, or for over five days. Different doses were used ranging from 3 μM to 25 mM in the food substrate. Simple activity was measured in the Trikinetics Drosophila Activity Monitor (DAMS), which measures photobeam breaks. Open field tests were performed by placing individual flies into 5 cm diameter wells bored into a plexiglass substrate with a transparent cover, arranged in an array of eight chambers. Flies were video recorded for ten minutes, and their activity scored by Noldus Ethovision XT software. Total distance, time in zone, and motion tracks were determined.
Results: For some drugs like cocaine, overall photobeam breaks increased dose-responsively over time for flies maintained on food + drug. Higher levels of the drug produced early lethality. Other drugs like theophylline did not produce an increase in general activity, even at high levels. In the open field tests, flies generally exhibited exploratory behavior that extinguished with time towards a baseline level of activity. Although cocaine produced an increase in overt activity, it led to a decrease in open-field activity compared to controls. For other drugs like theophylline, where there was no effect on overt activity, a significant increase in open-field activity was observed. There was no difference in the

time spent in the central zone between control flies and those fed cocaine. Flies fed theophylline, however, spent significantly less time in the central zone than controls, despite having higher open-field activity levels. Together, these data indicate that different stimulant drugs elicit different spectra of behavior. We believe that these can be exploited to elucidate molecular mechanisms unique to a particular drug.

Conclusions: The incorporation of open-field testing to the study of stimulant drugs in the fly provides more information regarding the effects of a drug than simple activity monitoring or subjective impairment scales. These expanded outcomes will allow for better discrimination of the effects of drugs that may otherwise appear to be overtly the same, allowing for more sophisticated discovery screens to be performed towards elucidating mechanisms underlying the effects of a particular drug. Studying these drugs in the fly, and harnessing their powerful genetics and ease of use as a model system, along with more effective tools, will ultimately lead to rapid and key fundamental discoveries that we can translate back to mammalian systems, and potentially to new therapies against drug abuse.

Keywords: Drosophila melanogaster, cocaine, open field, drug abuse, behavioral pharmacology

Disclosure: C. Nichols, Nothing to Disclose; I. Bruce, Nothing to Disclose; J. Becnel, Nothing to Disclose.

M124. Cortical Thickness, Regional Brain Volumes, and Symptom Severity in Body Dysmorphic Disorder

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Background: Body dysmorphic disorder (BDD) is characterized by preoccupation with perceived defects of appearance, causing significant distress and disability. Despite its prevalence (1-2% of the population) and severity, relatively little is known about the neurobiology. Only three small studies have previously investigated brain morphometry in BDD. Two of these found greater total white matter volume, but there were inconsistent abnormalities found in prefrontal and subcortical volumes. No study in BDD to date has investigated cortical thickness, which may provide a more sensitive measurement of subtle morphometric abnormalities. The purpose of this study is to investigate cortical thickness, as well as cortical, subcortical, and white matter volumes in a large cohort of individuals with BDD in comparison to healthy controls. An additional goal was to investigate correlations between these morphometric measures and clinical symptom severity and insight. Based on findings from previous morphometric studies, we hypothesized that individuals with BDD would have greater total white matter, abnormal prefrontal cortical thickness and volume, and abnormal subcortical volumes relative to healthy controls. We also hypothesized the BDD symptom severity would correlate with amygdala volume and inferior frontal gyrus thickness.

Methods: Participants: 85 medication-free, right-handed males and females with DSM-IV BDD (N = 44), and healthy controls (N = 41) of equivalent gender, age, and years of education participated. MRI: We acquired high-resolution structural MRI brain scans (T1-weighted MPRAGE, sagittal, 1x1x1 mm voxels) for each subject on either a Siemens Allegra (N = 49) or Trio scanner (N = 37). FreeSurfer (v5.0.0) was used to obtain cortical thickness at each vertex on the brain surface and volumes of cortical and subcortical structures (smoothing kernel = 15 mm). The automatically generated grey and white matter segmentations were quality controlled and manually edited with control points by a blinded researcher. Statistical Analysis: To test our hypotheses regarding regions of interest (ROI), we performed repeated measures ANOVA using PROC GLM in SAS. We used group (BDD or control), gender, scanner, and group*gender interactions as independent variables. Normalized volumes in thalamus, anterior cingulate cortex, orbitofrontal cortex, inferior frontal gyrus, total white matter, and caudate laterality quotient were the dependent variables. We performed a similar ROI analysis for cortical thickness, with the dependent variables being anterior cingulate cortex, orbitofrontal cortex, and inferior frontal gyrus. As an exploratory analysis, we used qdec in FreeSurfer to analyze cortical thickness at each vertex in the left and right hemisphere. We tested between-groups differences using the independent variables of group, scanner, and demeaned intracranial volume. In the BDD group, we performed separate linear regressions using the clinical variables of BDD-YBOCS (BDD symptom severity), BABS (measure of insight), and HAMA (anxiety), additionally covarying for scanner, demeaned intracranial volume, and gender. Cortical thickness and volumes were the dependent variables. Qdec results were corrected for multiple comparisons using the FDR method with a threshold of 0.05.

Results: The BDD group demonstrated significantly thinner cortex than healthy controls in a cluster in the right middle temporal gyrus, as revealed by the vertex-wise analysis. No significant group differences were evident for any of the *a priori* ROIs for volume or cortical thickness. Significant results were also found for the vertex-wide regression of BDD-YBOCS scores. Higher BDD-YBOCS scores, indicating more severe BDD symptoms, were associated with thinner cortical gray matter in clusters including the bilateral superior parietal cortex, bilateral pars triangularis, bilateral parahippocampal gyrus, left paracentral gyrus, right supramarginal gyri, left fusiform, and left medial orbitofrontal cortex.

Conclusions: This study represents the largest morphometric analysis in BDD to date. Compared to controls, BDD subjects had thinner cortex in the right middle temporal gyrus. In addition, more severe BDD symptoms were linked to thinner cortex in parietal, prefrontal, and temporal regions in both hemispheres. Some of these regions overlap with regions found to be functionally abnormal in previous visual processing functional neuroimaging studies. These results suggest that although subtle neuroanatomical abnormalities may exist in the brains of BDD subjects relative to healthy controls, clinical phenotypes may more sensitively map to brain pathophysiology than categorical diagnoses. A limitation of the study is the use of automated tissue segmentation and brain region parcellation software, which is not always accurate; however, quality control steps were taken to minimize errors. A strength of the study is that all subjects were medication-free, which is otherwise a common confound in many clinical studies that can potentially affect brain structure and function.

Keywords: body dysmorphic disorder, cortical thickness, brain volume, symptom severity, BDD-YBOCS

Disclosure: S. Madsen, Nothing to Disclose; T. Pirnia, Nothing to Disclose; A. Zai, Nothing to Disclose; T. Moody, Nothing to Disclose; J. Feusner, Nothing to Disclose.

M125. Compared to What? Reappraising the Early Brain Overgrowth Hypothesis in Autism

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Background: The presence of a robust association between Autism Spectrum Disorder (ASD) and early brain overgrowth (EBO) is widely accepted, prominent in lay understanding of the condition, and continues to influence the cutting edge of ASD research. The bulk of published data regarding early brain growth in ASD comes from studies of head circumference (HC), an excellent proxy for

brain size in early childhood. Two developments since last systematic review of EBO studies in ASD urge reappraisal of the evidence base behind the influential notion in ASD research however: (i) multiple independent studies outside the field of ASD research have found that large contemporary samples of typically developing children appear to show EBO relative to HC reference norms (HCRNs) that have been used by several seminal EBO reports in ASD, and (ii) multiple new longitudinal tests of the EBO hypothesis have been published which build on earlier work by using larger sample sizes and contrasting HC growth in ASD with locally recruited controls (LRCs) as well as HCRNs.
Methods: We systematically review all published HC tests of the EBO hypothesis in ASD (34 studies encompassing ~ 3k ASD and 60k LRC participants), and analyze new data from a cohort of 57 preschool-aged male Caucasian children (35 ASD, 22 LRCs) with ~ 330 longitudinal HC measures between birth and age 18 months. Our study (i) distinguishes cross-sectional analyses of mean HC in ASD within a given age-range from longitudinal analyses that can measure brain size change, and (ii) assesses the dependence of evidence for EBO on the type of control data with which HC data in ASD are compared. We supplement traditional sources of HCRNs in ASD research [such as the Center for Disease Control (CDC)] with recently published "Primary Care Norms" (PCNs): the largest (~ 500k HC measures between birth and 18 months) contemporary set of US-based HCRNs.
Results: Systematic Review: The majority (> 65%) of HC studies in ASD are cross-sectional. Of the 11 existent longitudinal HC studies, 10 have been published since the EBO hypothesis was last subjected to systematic review. 85% of all HC studies in ASD make use of HCRNs. Cross-sectional studies that do not find evidence for brain enlargement in ASD tend to include a comparison with LRCs rather than solely relying on HCRNs ($p = 0.0006$), and tend to use smaller age-ranges for calculating mean HC ($p = 0.03$). All published comparisons of HCRN-defined macrocephaly rate in ASD vs. LRC groups have been negative. Elevated macrocephaly rates in ASD relative to HCRN-defined null of 3% vary 6-fold, with older HCRNs tending to identify higher rates ($p = 0.04$). Over 85% of all longitudinal HC tests of the EBO hypothesis have compared ASD data to CDC HCRNs and identified rapid HC centile increases in ASD between birth and ~age 12 months. In contrast, most studies comparing HC growth between ASD participants and LRCs do not find evidence for EBO in ASD during the first year of life. By transforming existing HC reports into a common CDC reference frame we confirm the well-replicated pattern of EBO in ASD relative to CDC HCRNs, but show that the timing of this EBO is almost perfectly recapitulated by (i) 2012 PCN HCRNs which incorporate > 500k HC measures from typically developing children in the US, *and* (ii) a weighted mean summary of HC growth for all LRCs included in ASD research. New Data Analysis: We did not find any cross-sectional differences in raw HC between ASD and LRCs at any pediatric surveillance time-point in the first 24 months of life, or group differences in raw HC change between time-points. Both ASD and LRC groups showed abnormally accelerated HC growth relative to CDC HCRNs, but had a stable mean PCN HC centile that remained within normal ranges between birth and 24 months.
Conclusions: By combining systematic review with analysis of new data and comparisons across multiple HCRNs we find several lines of evidence that oppose the hypothesis of EBO in ASD as currently formulated. Specifically (i) EBO in ASD relative to CDC appears to reflect a mis-match between CDC norms and contemporary patterns of HC growth that is shared by large samples of healthy children, (ii) HCRN-defined macrocephaly rates in ASD have usually been indistinguishable from those in contemporaneously ascertained LRCs, and (iii) macrocephaly rate reports that lack parallel LRC comparison appear to vary as a function of how old the HCRNs used define macrocephaly were. Existing data potentially provide partial support for a subtle divergence of HC growth between a sub-group of children with ASD and LRCs during the second year life that (i) results in ~5 mm group difference in mean HC at 24 months, and (ii) may index body size and SES related confounds.
Keywords: Autism Brain Overgrowth Head Circumference Systematic Review
Disclosure: A. Raznahan, Nothing to Disclose; R. Lenroot, Nothing to Disclose; A. Thurm, Nothing to Disclose; M. Gozzi, Nothing to Disclose; S. Spence, Nothing to Disclose; S. Swedo, Nothing to Disclose; J. Giedd, Nothing to Disclose.

M126. Nicotinic-Mediated Effects on Brain Reward Function Are Modulated by α5-Containing Nicotinic Acetylcholine Receptors

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Background: Increased risk of tobacco addiction in humans has been repeatedly associated with allelic variation in the *CHRNA5* gene, which encodes the α5 nicotinic acetylcholine receptor (nAChR) subunit. Recent studies have begun to elucidate the role of this nAChR subunit in the motivational properties of nicotine, the major addictive agent in tobacco smoke. Indeed, we found that α5-containing nAChRs in the medial habenulo-interpeduncular pathway exert an inhibitory influence on nicotine intake, particularly at high doses of the drug. Here, we sought to extend these findings by assessing whether α5-containing nAChRs alter brain reward function with acute or chronic nicotine administration and during antagonist-precipitated or spontaneous withdrawal.
Methods: Mice with null mutation of the α5 nAChR subunit gene (*Chrna5*) and their wildtype littermates were implanted with cranial electrodes and trained in a discrete-trial current-threshold intracranial self-stimulation (ICSS) procedure until stable reward thresholds were obtained. Mice were then injected with varying doses of nicotine (0, 0.03125, 0.0625, 0.125, 0.25, 0.5 mg/kg, free-base, subcutaneous) according to a Latin square design. Thereafter, minipumps delivering either saline or nicotine (24 mg/kg/day) were implanted subcutaneously. To assess withdrawal-associated reward deficits, mice were first administered the general nAChR antagonist, mecamylamine (5 mg/kg, i.p.). Next, they were permitted to reestablish stable levels of ICSS responding, followed by surgical removal of the minipumps to assess spontaneous withdrawal.
Results: Wildtype mice exhibited an 'U' shaped dose response curve, with maximal reward threshold lowering at 0.0625-0.125 mg/kg of nicotine. The α5 knockout mice displayed a similar lowering at these doses, but also maintained lowered threshold levels at higher doses. Following implantation of the saline or nicotine minipumps, all groups maintained baseline threshold levels across seven days of exposure. Mecamylamine-precipitated withdrawal elicited significant increases in threshold levels for wildtype mice implanted with nicotine, but not saline, minipumps. Intriguingly, α5 knockout mice implanted with saline or nicotine minipumps both demonstrated increased threshold levels following mecamylamine administration. Finally, after minipump removal, nicotine-treated wildtype mice exhibited an increase in threshold levels compared to saline-treated mice consistent with spontaneous withdrawal, whereas the α5 knockout mice treated with saline or nicotine did not differ from baseline levels.
Conclusions: These data demonstrate that α5-containing nAChRs modulate nicotine's effects on brain reward function. Consistent with our prior findings in rats, deficient expression of α5 nAChR subunits extends the range of doses that elicit rewarding effects, as evidenced by decreased ICSS thresholds. During nicotine withdrawal, α5 knockout mice do not appear to display reward deficits compared to saline controls. Together, these findings reveal fundamental insights into the mechanisms underlying nicotinic

modulation of brain reward function and are of high relevance to the human condition given that receptor function is globally disrupted in both the knockout mice and human allelic carriers. Therefore, these data will likely have clinical and therapeutic implications for smoking cessation. *Supported by the National Institute on Drug Abuse (DA026693 and DA032543 to CDF; DA020686 to PJK)*

Keywords: addiction, nicotinic acetylcholine receptor, α5, Alpha 5 subunit

Disclosure: C. Fowler, Nothing to Disclose; P. Kenny, Nothing to Disclose.

M127. Targeted Deletion of the α2 Nicotinic Acetylcholine Receptor Subunit Gene (Chrna2) Potentiates Sexual Dimorphism in Emotional Processing

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Background: The alpha(α)2 nicotinic acetylcholine receptor (nAChR) subunit gene (*Chrna2*) has restricted expression within limbic brain regions, including the neocortex, hippocampus and amygdala. Targeted deletion of the *Chrna2* gene induces an absence of nicotine facilitation and depression of hippocampal long-term potentiation in $Chrna2^{-/-}$ mice. To test whether nicotine mediated hippocampal memories are behaviorally modified in $Chrna2^{-/-}$ mice, current studies observe mice through a pre-exposure facilitation of fear conditioning task.

Methods: Male and female $Chrna2^{+/+}$ and $Chrna2^{-/-}$ adult mice were initially handled for three days. Subsequently, mice were administered a pre-training injection of vehicle or nicotine (0.09 mg/kg, i.p.) and pre-exposed to a novel contextual chamber for 10 minutes. The subsequent day, mice were placed in the same contextual chamber and within 10 seconds of entry given a single mild electric shock (0.75 mA, 2 sec). On the third day, animals were administered a pre-testing injection of vehicle or nicotine (0.09 mg/kg, i.p.), and assessed for freezing behavior in the shock exposed environment for 8 minutes. Extinction behavior was then assessed for up to 5 weeks after testing in the same contextual chamber for 20 min/session. Freezing behavior, measured as an absence of movement, was monitored through near infrared lighting and a digital video camera placed in front of the contextual chambers, and scored through a Med Associates fear conditioning software package.

Results: Results illustrate sexually dimorphic behavioral consequences to targeted deletion of the *Chrna2* gene. Independent of nicotine treatment, $Chrna2^{-/-}$ male mice (vs. wildtype) have reduced freezing behavior on the day of contextual testing. On the other hand, nicotine treated $Chrna2^{-/-}$ female mice illustrate a failure to extinguish freezing behavior for up to 5 weeks post-contextual testing.

Conclusions: The results provide evidence for an important role for the *Chrna2* gene in modulating emotional memory processing in key limbic brain regions in both male and female mice, with selective effects of nicotine treatment based on sex and genotype. Future studies are needed to address the mechanisms mediating these sexually dimorphic effects. Furthermore, translational studies are needed to assess whether rare and/or functional single nucleotide polymorphisms in the coding region of the *Chrna2* gene modulate nicotine-facilitated emotional memories in humans, paying special attention to gender selective effects. Such studies could provide critical mechanisms mediating cognitive emotional processing disorders during adolescence and adulthood, highlight a potential novel genetic target mediating individual susceptibility to these deficits, and provide a possible site for therapeutic intervention.

Keywords: Nicotine, Emotional Memory, Nicotinic Receptors, Hippocampus

Disclosure: S. Lotfipour, Nothing to Disclose.

M128. Serotonin Transporter Deficient Rats Exhibit Enhanced Acquisition and Disrupted Extinction of Conditioned Fear

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Background: Human carriers of the short-allele of the serotonin transporter gene-linked polymorphic region (SERT-LPR) have reduced SERT binding and expression and a higher incidence of anxiety/depression-related traits when combined with environmental adversity during development. This lower SERT expression also appears to facilitate amygdala excitation, which suggests these patients may be vulnerable to post traumatic stress disorder (PTSD). Since conditioned fear test is a well-established model of cue associated fear memories (a cardinal symptom of PTSD), we used rats that have homozygous and heterozygous nullmutations of SERT gene to test our hypothesis that deletion of SERT genes in rats will have gene-dose dependent changes in their ability to acquire conditioned fear (i.e., phobia) and/or their subsequent ability to extinguishsuch acquired fear responses.

Methods: Utilizing a standard conditioning chamber (Hamilton-Kinder) three genotypes of rats (WT, SERT+/−, SERT−/−) went through 5 days of experimentation: Day 1 Habituation; Day 2 Conditioning; Day 3 Fear recall testing; Day 4 Extinction Training; and Day 5 Extinction Recall Test.

Results: Compared to wild type rats (SERT+/+), only SERT−/− rats showed increased acquisition of conditioned fears. However on subsequent days of testing both SERT−/− and SERT−/+ rats showed increased expression of conditioned fears compared to SERT+/+ rats. Currently experiments are in progress that will determine the extent to which SERT genotype, and genotype X fear conditioning effect: 1) amygdala gene expression using focused RT-PCR arrays (Taqman Low Density Arrays); and 2) neurochemical systems in the amygdala utilizing patch clamp studies in slice preparations.

Conclusions: Rats that have homozygous and heterozygous null mutations of SERT gene showed significantly delayed extinction of conditioned fear further supporting the notion that reduced SERT function is a genetic risk for developing chronic anxiety, phobias and PTSD.

Keywords: Fear, PTSD, amygdala, serotonin, anxiety

Disclosure: P. Johnson, Nothing to Disclose; S. Fitz, Nothing to Disclose; A. Molosh, Nothing to Disclose; W. Truitt, Nothing to Disclose; A. Shekhar, **Part 1:** Dr. Shekhar has conducted contractual work in collaboration with Eli Lilly & Co. and Johnson & Johnson & Co. over the last two years which is unrelated to the results presented on the poster submission.

M129. Exposure to Chronic Stress During Pregnancy Prevents the Beneficial Effects of Motherhood on Dendritic Spines in the Hippocampus and Medial Prefrontal Cortex

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Background: Exposure to chronic stress in pregnancy is a known risk factor for postpartum depression and anxiety. However, very little is known about the underlying neural mechanisms by which pregnancy stress increases susceptibility to postpartum mental illness. Here, we investigated the possibility that pregnancy stress compromises postpartum structural plasticity in the hippocampus and prefrontal cortex, directly connected brain regions that have been implicated in mood disorders.

Methods: Pregnant Sprague-Dawley rats were exposed to 20 min of inescapable swim stress twice daily from gestation day (GD) 7-13 and 30 min of restraint stress twice daily from GD 14-20. Following birth (postpartum day 0; PD 0), postpartum females subjected to

pregnancy stress were left undisturbed until PD 21-22 at which time they were sacrificed along with postpartum females that did not undergo pregnancy stress and non-stressed virgins. DiI labeling and confocal microscopy was used to analyze dendritic spine density on apical and basal dendrites of pyramidal neurons in area CA1 of the ventral hippocampus and layer 2/3 pyramidal neurons in the medial prefrontal cortex (mPFC).
Results: Validating the stress procedure, we demonstrated decreased body weight gain, increased adrenal weight, and impaired maternal behavior in stressed mothers relative to their non-stressed controls. Compared to virgin females, non-stressed postpartum females had a greater number of dendritic spines on apical and basal dendrites of pyramidal neurons in layer 2/3 of the mPFC and in the CA1 region of the ventral hippocampus. In both the mPFC and ventral hippocampus, chronic pregnancy stress abolished the motherhood-induced increase in dendritic spine density.
Conclusions: We show that chronic pregnancy stress prevents neuronal growth in the hippocampus and mPFC during the postpartum period. These results suggest that stress experienced during pregnancy may enhance susceptibility to postpartum mental illness by interfering with structural plasticity in brain regions regulating mood.
Keywords: postpartum, depression, anxiety, maternal, prenatal stress
Disclosure: B. Leuner, Nothing to Disclose; P. Fredericks, Nothing to Disclose; C. Nealer, Nothing to Disclose.

M130. Cortisolemia, Psychopathology and Treatment Response in First-episode Schizophrenia

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Background: For a decades it has been theorized that stress sensitivity and activity of the hypothalamic pituitary adrenal axis (HPA), as indexed by cortisol secretion, may be relevant to development and expression of schizophrenia. Cortisol secretion is an easily assayed biological correlate of the stress sensitivity. The aim of this study was to assess cortisolemia including Dexamethasone Suppression Test (DST) and its clinical correlates during the acute treatment in first-episode schizophrenia patients.
Methods: Males, consecutively hospitalised for the first time with first-episode schizophrenia (according to ICD-10) at the Department of Psychiatry in Brno, who underwent DST on admission (baseline) and at discharge (final) and who provided written informed consent, were included. Nonsupression was defined as less than 50% decrease of cortisolemia after 1 mg dexamethasone. The psychopathology of the patients was evaluated using the Positive and Negative Syndrome Scale (PANSS). At the end of acute treatment responders (minimally 30% decrease of the total PANSS score (delta PANSS) and nonresponders were identified. All patients were treated openly with the second-generation antipsychotics. Risperidone was used as the first choice treatment.
Results: A total of 158 males were included. Mean age was 23.6 (± 5.5), mean duration of illness 5.9 (± 8.9) months. The short-term acute treatment led to a significant decrease in cortisolemia and rates of nonsupression The mean baseline cortisolemia was 265,5 (± 156.3), postdexamethasone cortisolemia (at 4 p.m.) was 69.8 (87.1) mMol/l. At the end of the acute treatment the values were 183.9 (± 96.3) respective 28.8 (± 45.2). 29/ 158 (18%) of patients were DST nonsuppressors at medication-free baseline, 10/158 (6%) after acute treatment. At the end of acute treatment 121/158 (77 %) of patients fulfilled the criteria for the treatment response. No significant correlations were found between cortisolemia, the total PANSS and all the PANSS subscales scores or delta PANSS at any time points. There was no significant difference in pre- and postdexamethasone cortisolemia and in the rate of nonsupression between responders and nonresponders. However, final nonsupressors had a significantly higher total PANSS and general subscale PANSS scores at baseline. Initial nonsupressors had a significantly lower delta negative subscale score, e.g. worse response to treatment. Concerning individual PANSS items, a significant negative correlation (after Bonferroni corections, $p = .002$) was found between halucinatory behaviour at the end of acute treatment and baseline cortisolemia and between halucinatory behaviour at the beginning and final postdexamethasone cortisolemia. Further, disturbance of abstract thinking at the end of the treatment correlated positively with final postdexamethasone cortisolemia. This partially corresponds with the worse treatment response to negative symptoms of schizophrenia.
Conclusions: Generally hypercortisolemia is considered to be a reflection of the rate of stress associated with experiencing psychotic symptoms. Our data are in agreement with studies which pointed out that there is an association between DST nonsuppression and negative symptoms of schizophrenia. Also in our previous publication concerning this topic in a much smaller sample we found a significant correlation between negative symptoms (negative PANSS subscale score) and postdexamethasone cortisolemia after 6 weeks of antipsychotic treatment. We may speculate that there are two groups of patients – the first one with stress-related DST nonsuppression and the second group with DST nonsupression associated with primary negative symptoms and eventually structural abnormalities with lower treatment response to antipsychotics. In conclusion DST test is simply applicable and may be helpful to clinicians in identification of patients with worse influencing of negative symptoms of schizophrenia. Supported by the project CEITEC – Central European Institute of Technology (CZ.1.05/1.1.00/02.0068) from European Regional Development
Keywords: dexamethasone supression test, cortisolemia, first-episode schizophrenia, psychopathology, response
Disclosure: E. Ceskova, Nothing to Disclose; R. Prikryl, Nothing to Disclose.

M131. Trauma Timing Predicts Variation in PTSD-related Outcomes

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Background: Posttraumatic stress disorder (PTSD) is characterized by states of hyperarousal, episodes of re-experiencing the trauma, and avoidance of trauma-related reminders. These behavioral changes are associated with disruptions in various domains of physiology including the neuroendocrine and autonomic nervous systems and alterations in brain volume, as well as reduced psychosocial functioning. While most studies have shown elevated autonomic nervous system (ANS) arousal among individuals diagnosed with PTSD, both resting and in response to challenge, there are a number of discrepancies between PTSD studies examining other physiological measures. Some PTSD studies show lower basal levels of cortisol and a reduced cortisol response to challenge paradigms compared to controls, while other studies report higher cortisol levels and a hyperactive response to challenges. Some studies, but not all, have reported a reduction in hippocampal volume bilaterally or in the left or right hippocampi in individuals with PTSD compared to controls. Many of these neuroendocrine, brain volume and ANS changes are also found in adults with histories of exposure to childhood trauma. Thus, it is plausible that trauma experienced in early life in particular may lead to a specific constellation of physiological and behavioral symptoms, and that differences in timing may explain some of the discrepancies in the PTSD literature. The current study

explores the impact of timing of the trauma on HPA axis function, brain volume, autonomic arousal, and PTSD symptoms in a sample of individuals diagnosed with comorbid PTSD and alcohol dependence. Since 50% of individuals diagnosed with PTSD also have a diagnosis of alcohol dependence, the present study not only contributes to the knowledge base of PTSD, but also adds to the small but important literature on these comorbid disorders.
Methods: Participants included 51 treatment-seeking inpatients diagnosed with PTSD and comorbid alcohol dependence (AD) who were hospitalized at the National Institutes of Health Clinical Center for four weeks. Participants ranged in age from 21 to 50 years ($M = 40.80$, $SD = 8.17$); 43% were female and 43% were Caucasian. There were 43 in the child trauma (CT) group and eight in the adult trauma (AT) group. Groups did not significantly differ on demographics or alcohol-related measures. Three weeks after abstinence, they underwent a combined Trier Social Stress Test and Cue Reactivity challenge (Trier/CR) followed by structural magnetic resonance imaging (MRI) a week later. Outcomes of interest included neuroendocrine (ACTH and cortisol) and autonomic (heart rate (HR)) response to the challenge, hippocampal brain volume, and measures of PTSD symptom severity. Repeated measures analysis of covariance and one-way analysis of variance models were used to explore differences in outcomes as a function of trauma timing (childhood vs. adulthood).
Results: Individuals in the CT group had significantly lower cortisol responses to the Trier/CR compared to those in the AT group (F (1,41) = 5.47, $p = 0.02$). The CT group had a smaller right (Rt) hippocampal volume than the AT group (F (1,41) = 4.91, $p = 0.033$), and higher arousal symptoms throughout the hospitalization (F (1,42 = 8.40, $p = 0.006$). The CT group also evidenced a trend for higher basal HR and higher HR during the Trier/CR. There were no between group differences in left hippocampal volume, overall PTSD symptom severity, or PTSD-related avoidance or re-experiencing.
Conclusions: Individuals with CT compared to those with AT, have a reduced cortisol response to the stress of the Trier/CR, a reduced Rt hippocampal volume, and a higher autonomic arousal, suggesting that timing of trauma may be an important determinant for the physiological functioning of individuals with comorbid PTSD and AD. The importance of these findings is supported by various preclinical and clinical studies suggesting that CT may contribute to the biological programming of the HPA axis. Potential mechanisms of this programming include epigenetic changes involving the glucocorticoid receptors, which have been shown to occur in both rats and humans exposed to early life trauma. Prospective longitudinal studies are needed to elucidate the mechanisms by which CT contributes to PTSD.
Keywords: PTSD Cortisol Hippocampus alcohol
Disclosure: D. George, Nothing to Disclose; L. Kwako, Nothing to Disclose; K. Garg, Nothing to Disclose; J. Sells, Nothing to Disclose; E. Grodin, Nothing to Disclose; M. Schwandt, Nothing to Disclose; D. Hommer, Nothing to Disclose; M. Heilig, Nothing to Disclose.

M132. The Relationship between Sleep Quality AND Morning/Eveningness, Seasonality, Activity Levels, and Dim Light Melatonin Onset in Depressed Patients with Bipolar Disorder

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Background: Patients with Bipolar Disorder (BD) may be vulnerable to environmental cues which alter circadian rhythms and trigger mood episodes. Preliminary data from our group and others suggested that patients with bipolar depression can respond robustly to bright light therapy. Remission may be related to normalized sleep and other improvements in circadian timing. In this study, we examined the relationship between night-time sleep quality and the preference for morning or evening, seasonal variation in depressive symptoms and activity levels during the day and at night in depressed patients with Bipolar Disorder (BD). Also we explored dim light melatonin onset (DLMO) in BD patients with a current major depressive episode. We hypothesized that reduced sleep quality is associated with an evening preference, increased seasonality and reduced daytime activity levels in depressed bipolar patients.
Methods: Study patients were enrolled in the ongoing parent investigation to explore the efficacy of bright light therapy vs inactive comparator for bipolar depression: a randomized control trial (PI: D Sit; K23 Career Development Award - K23MH082114). We obtained assessments at baseline, prior to randomization. We confirmed the diagnosis of BD Type I or II and a current major depressive episode (duration >4 weeks) with the Semi-Structured Clinical Interview for the DSM-IV. We assessed sleep quality with the Pittsburgh Sleep Quality Index (PSQI; Buysse et al, 1989), morning/evening preferences with the Morningness Eveningness Questionnaire (MEQ; Horne and Östberg, 1976), seasonality with the Personal Inventory for Depression and Seasonal Affective Disorder (PIDS; Terman et al, 1998) plus the component Global Seasonality Score (GSS) to quantify the seasonal variation in the mood and energy of depressed patients (Rosenthal et al, 1987); and one-week activity levels with the Respironics actiwatch (model#AW16 and AWLP, Mini-Miller Co, Inc). From each patient, we collected 9 serial evening salivary melatonin samples to estimate DLMO.
Results: We included 16 women and 7 men ranging in age from 18-66 (mean = 47.7) years; 17 pts had BD-I and 6 had BD-II. The racial distribution comprised of 18 - white, 4 – African American and 1 – multiracial. For sleep quality (PSQI), 20 patients scored >5 and 14 scored >7. On the MEQ, 17 patients had an intermediate preference; 4 preferred the morning (3-moderately, 1-definitely) and 2 preferred the evening (moderately only). The association between sleep quality and MEQ was not significant [estimate of slope of the regression = 0.01899, 95% confidence interval-CI (-0.190, 0.229), p-value = 0.8526]. On the PIDS-GSS, 10 patients reported increased, 5 – moderate and 8 – no seasonal component to their depressive symptoms. Analysis of variance indicated a significant association between sleep quality and seasonality ($p = .0333$). Sleep duration from the self reported PSQI and the actigraphs was consistent. There is a significant linear association between sleep quality and wakeful activity [linear regression estimate = -0.0107, 95% CI (-0.0219, 0.0005), for $p = 0.10$ level only; $p = .0594$]. To characterize evening melatonin release, we compared the cut-point (conventional) and variance (novel) methods for onset time and amplitude. Patients with early (phase-advanced) vs late (phase delayed) DLMO had significant differences in age and bedtime. Gender, BD I vs II, sleep quality and sleep duration did not differ significantly between patients with early vs late DLMO.
Conclusions: Patients with bipolar depression may have an increased or moderate seasonal component to their depressive symptoms. Sleep duration may be estimated with the PSQI or actigraphy. Even during a depressive episode, bipolar patients with better sleep quality tend to be more active during the day. Increased daytime activity likely is related to improved physical health. We will discuss our approach to refine the collection methodology and statistical analysis of the DMLO data. Future research on new bipolar treatments may include measures to assess sleep parameters as possible clinical markers of illness, treatment response and physical health.
Keywords: Bipolar Disorder, Depression, Sleep, Seasonality, Melatonin
Disclosure: D. Sit, Nothing to Disclose; C. Wiltrout, Nothing to Disclose; E. Fowler, Nothing to Disclose; M. Mitchell, Nothing to Disclose; K. Wisner, Nothing to Disclose; H. Seltman, Nothing to Disclose.

M133. Brief Cognitive Intervention Can Modulate ACTH Response to the Trier Social Stress Test

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Background: Stress undermines health, perhaps via activation of the HPA axis. There is evidence that psychological factors (i.e., sense of control, familiarity, effective coping, and social support) can buffer stress effects and HPA axis activation. There is also evidence that a compassionate goal orientation (striving to help others rather than promoting the self) is associated with health and well-being, perhaps via HPA buffering effects. We utilized a laboratory model of social evaluative threat (Trier Social Stress Test, TSST) to activate the HPA axis and study the stress buffering effects of control, familiarity/coping, and compassionate goals. ACTH responses have now been analyzed and mirror previously reported cortisol findings.
Methods: Healthy participants ($n = 54$) were exposed to a TSST after receiving standard instruction (SI) or one of three intervention instructions (access to "Control" over threat exposure, cognitive intervention to increase familiarity and effective "Coping," or a "Compassion" intervention designed to shift goal orientation from self-promotion to helping others).
Results: Instruction type significantly altered ACTH responses over the course of the TSST ($p < .0001$), largely due to stress buffering effects of the "Compassion" intervention. "Control" alone had no impact relative to SI, yielding identical ACTH responses over time ($p = .73$). "Coping" actually raised baseline ACTH levels relative to the other groups ($p's < .02$), without substantially reducing ACTH responses to the stressor. However, the "Compassion" intervention significantly reduced ACTH peak levels and responses to stress ($p = .02$).
Conclusions: ACTH results mirror previously reported findings with cortisol responses to the TSST: "Coping" instructions increased anticipatory stress and did not reduce the stress response, whereas "Compassion" instructions (designed to shift the usual self-promotion goal orientation of the TSST to an altruistic, helping others goal orientation) significantly reduced stress responses to this stressor. Re-orienting cognitive set away from self-protection/self-promotion and towards helping others may provide a particularly potent way to reduce the stress created by social evaluative threat.
Keywords: stress, ACTH, TSST, coping, compassion
Disclosure: S. Mayer, Nothing to Disclose; T. Erickson, Nothing to Disclose; H. Briggs, Nothing to Disclose; J. Crocker, Nothing to Disclose; I. Liberzon, Nothing to Disclose; J. Abelson, Nothing to Disclose.

M134. The Novel Vasopressin V1a Receptor Antagonist SRX246 Blocks Vasopressinergic Modulation of Emotion: An fMRI Study

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Background: Preclinical models of affective disorders have identified an important role for central vasopressinergic signaling. Clinical studies indicate that stress related psychiatric disorders involve dysregulation of vasopressin signaling, but this knowledge has not resulted in a therapeutic approach that modifies brain vasopressin function. The study objective was to demonstrate SRX 246, a novel, small molecule vasopressin receptor antagonist with high specificity and selectivity for the V1a receptor, has centrally effects in humans. The experimental approach utilized challenge with intranasally administered vasopressin combined with a social neuroscience task in the fMRI scanner as a probe of central vasopressin receptor function.
Methods: The study utilized a double-blinded, randomized design to compare the effects of chronic SRX246 versus placebo on vasopressinergic modulation of brain response to affective stimuli (emotional faces) as measured by fMRI combined with acute intranasal vasopressin challenge. 29 healthy, medication free male adults were randomized to 5-10 days of SRX246 (120 mg BID) or placebo. fMRI BOLD response to angry faces versus control (Fixation cross) was measured at baseline and after 5 - 10 days of oral drug, 45 minutes after randomized, double-blind intranasal administration of 40 IU vasopressin or placebo. To test the primary hypothesis, BOLD signal intensity in regions of interest (ROIs) was analyzed by Repeated Measures ANOVA. Post-drug session data were compared between groups to examine main effects of drug using ANCOVA, covarying for baseline signal intensity in ROIs.
Results: Consistent with previous Phase I results, SRX246 was well tolerated by all study subjects. Repeated Measures ANOVA of extracted ROIs of the left amygdala detected a significant interaction of oral and intranasal drug in contrasts of Angry vs. Fixation. Follow up ANOVA of change scores (Session 2 – Baseline) revealed that SRX246 blocked the effect of vasopressin on the accommodation of neural response to Angry faces. Main effects for SRX246 were found in the left and right temporoparietal junction (BA39) and anterior cingulate.
Conclusions: Confirmatory evidence for a central effect of SRX246 was found: SRX246 blocked exogenous vasopressin effects on amygdala reactivity to emotional stimuli over the two study sessions. Additional main effects of SRX246 were found in frontal and parietal brain regions that are part of a social and emotional processing circuit. This is the is the first study to show evidence than an orally administered small molecule vasopressin 1a receptor antagonist is able to block central effects of exogenously administered vasopressin in humans, and provides proof of concept for further development of SRX246 to treat stress related neuropsychiatric disorders.
Keywords: vasopressin stress V1a-receptor fmri intranasal
Disclosure: R. Lee, **Part 4:** The research was funded by Azevan Pharmaceuticals; E. Coccaro, **Part 1:** Dr. Coccaro is a scientific advisor for Azevan Pharmaceuticals; R. McCarron, Nothing to Disclose; V. Towle, Nothing to Disclose; S. Lu, **Part 1:** Dr. Lu is employed by Azevan Pharmaceuticals, **Part 2:** Azevan Pharmaceuticals; C. Guillon, **Part 1:** Dr. Guillon is employed by Azevan Pharmaceuticals, **Part 2:** Azevan; K. Fabio, **Part 1:** Dr. Fabio is employed by Azevan Pharmaceuticals, **Part 2:** Azevan Pharmaceuticals; M. Brownstein, **Part 1:** Michael Brownstein is employed by Azevan Pharmaceuticals, **Part 2:** Azevan Pharmaceuticals, **Part 3:** Azevan Pharmaceuticals; N. Simon, **Part 1:** Neal Simon is employed by Azevan Pharmaceuticals, **Part 2:** Azevan Pharmaceuticals, **Part 3:** Azevan Pharmaceuticals.

M135. Antidepressant Efficacy of Ketamine in Treatment-Resistant Major Depression: a Two-Site, Randomized, Parallel-Arm, Midazolam-Controlled, Clinical Trial

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Background: Several small, single-site studies or case series in unipolar and bipolar depression have suggested that ketamine – a glutamatergic NMDA receptor antagonist – may be associated with a rapid antidepressant effect. Previous controlled trials of intravenous (IV) ketamine utilized cross-over designs with saline

as the control condition. This methodological feature has raised concerns over the quality of the blind, given ketamine's well-known psychoactive effects, and consequently has limited our ability to draw firm conclusions regarding the antidepressant efficacy of ketamine. In the current study the investigators have conducted a two-site, randomized, controlled, parallel-arm clinical trial of IV ketamine versus IV midazolam in treatment-resistant unipolar major depression (TRD). Midazolam – a short-acting, water soluble benzodiazepine – was selected as an "active" control condition due to its potential to mimic many of the transient psychoactive effects associated with ketamine. Here we report top-line results from the largest clinical trial of ketamine in major depression conducted to date.

Methods: Following a washout of concomitant antidepressants or other psychotropic medication, 73 patients, ages 21-80, with unipolar major depressive disorder, without psychotic features, and moderate-to-severe depressive symptoms (IDS $\geq$ 32), were eligible for randomization. All patients were treatment-resistant, defined as a failure to response to at least three antidepressant trials with adequate doses and duration. Patients were randomized to receive a single 40-minute IV infusion of either ketamine hydrochloride (0.5 mg/kg) or midazolam (0.045 mg/kg) in a 2:1 randomization scheme. 72 individuals comprised the intent-to-treat (ITT) sample and received study drug under triple-blind conditions under the supervision of an anesthesiologist masked to drug identity along with the patient and rater. Patients were discharged 24 hours following the infusion, and received follow-up outpatient evaluations at 48 hr, 72 hr, and 7 days post-infusion, while remaining antidepressant medication-free. The primary outcome was change in MADRS score from baseline to 24 hours post-infusion and proportion of participants meeting response criteria at 24 hours, defined as $\geq$ 50% reduction in MADRS score. Secondary outcomes included the (1) durability of antidepressant benefit over the subsequent 7-day interval and (2) safety and tolerability of the interventions.

Results: 52% of the ITT sample was female with a mean age was 45.5 ± 12.4 years. On average, patients had been ill for more than 20 years with an age of onset of the first major depressive episode of 21.0 ± 9.6 years and a length of current episode of 11.7 ± 13.4 years. Nearly one in three patients had experienced a prior suicide attempt (32.9%). The baseline IDS-C_{30} and MADRS scores were 48.1 ± 9.0 and 32.07 ± 5.9, respectively. After controlling for site differences, treatment, and time, a treatment x time interaction demonstrated differential change for the two groups over the first 24 h period ($F(1,70) = 9.62$, $p \leq 0.003$). Ketamine demonstrated a 16.5 point decrease ($t(46) = -10.31$, $p \leq 0.0001$) on the MADRS while midazolam showed an 8.8 point decrease ($t(24) = -4.63$, $p \leq 0.0001$). At 24 hours post-infusion, the response rate in the ketamine arm was 63.8%, compared to 28.0% in the midazolam arm ($p = 0.006$). Controlling for site differences, ketamine increased the odds of responding by a factor of 2.16 (95% CI 1.31-4.09). The remission rate at 24 hours in the ketamine arm was 36.2%, compared to 8.0% in the midazolam arm ($p \leq 0.011$). Controlling for site differences, ketamine increased the odds of remitting by a factor of 2.58 (95% CI 1.15-8.14). At Day 7, the response rate in the ketamine arm was 45.7%, compared to 18.2% in the midazolam arm ($p \leq 0.034$). After controlling for site differences, ketamine increased the odds of responding by a factor of 1.97 (95% CI 1.01−4.34). Remission rate at Day 7 was 34.8% and 18.2% for ketamine and midazolam respectively ($p \leq 0.26$). Controlling for site, ketamine increased the odds of remission by a factor of 1.93 (95% CI 0.94−4.79) relative to midazolam. Both study drugs were well-tolerated; ketamine was associated with a higher incidence of elevations in blood pressure compared to midazolam during the infusion period.

Conclusions: In the largest clinical trial testing the efficacy of IV ketamine in mood disorders conducted to date, ketamine was associated with a rapid and large antidepressant effect at 24 hours, significantly superior to midazolam, and this superior efficacy was maintained seven days post-infusion. Ketamine appears to possess rapid antidepressant effects independent of its transient psychoactive effects – a conclusion validated by the novel use of midazolam as an active control condition in this study. Hemodynamic alterations associated with ketamine were observed in some patients, emphasizing the importance of appropriate cardiorespiratory monitoring during this procedure.

Keywords: ketamine, antidepressant, major depression, treatment resistant, glutamate

Disclosure: J. Murrough, **Part 1:** Dr. James Murrough is a fulltime employee of Mount Sinai Medical Center and receives research mentoring from Dr. Dennis Charney, Dean of Mount Sinai School of Medicine. Dr. Charney has been named as an inventor on a use-patent of ketamine for the treatment of depression. If ketamine were shown to be effective in the treatment of depression and received approval from the Food and Drug Administration for this indication, Dr. Charney and Mount Sinai School of Medicine could benefit financially. This conflict is currently being managed by the Mount Sinai School of Medicine Financial Conflict of Interest Committee, **Part 4:** In the past two years, Dr. James Murrough has served as a site PI on industry-sponsored clinical trials involving the following companies: Evotec, Janssen Pharmaceuticals; D. Iosifescu, **Part 1:** CNS Response, Inc – consultant, **Part 4:** Since 2010 Dr. Iosifescu has received grant/research support through Mount Sinai School of Medicine from Brainsway, Euthymics Bioscience Inc, Neosync and Shire. In the next two years it is likely he will receive grants from Hoffmann-La Roche Inc and Astrazeneca LP; L. Chang, Nothing to Disclose; R. Al Jurdi, Nothing to Disclose; C. Green, Nothing to Disclose; S. Iqbal, Nothing to Disclose; S. Pillemer, Nothing to Disclose; A. Perez, Nothing to Disclose; A. Foulkes, Nothing to Disclose; A. Shah, **Part 1:** Astrazeneca; Bristol-Myers Squibb, **Part 4:** Bristol Myers Squibb; Johnson & Johnson; D. Charney, **Part 1:** Dr. Charney and Mount Sinai School of Medicine have been named on a use patent application of Ketamine for the treatment of depression. If Ketamine were shown to be effective in the treatment of depression and received approval from the Food and Drug Administration (FDA) for this indication, Dr. Charney and Mount Sinai School of Medicine could benefit financially, **Part 4:** None; S. Mathew, **Part 1:** Allergan; Bristol-Myers Squibb; Cephalon, Inc; Corcept; Noven; Roche; Takeda, **Part 4:** Bristol Myers Squibb; Johnson & Johnson

M136. Can Prediction of Psychosis be Improved? Findings from the 12-year Recognition and Prevention (RAP) Program

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Background: Over the past decade, considerable interest has been directed to the possible prevention of psychosis, but predictive accuracy has been challenging, and the success of treatment interventions limited. Individuals considered to be in the prodromal phase are typically referred to as being at clinical high-risk (CHR), since elevated risk (broadly ranging from about 20 to 40% in the literature) is based on the emergence of subtle or attenuated positive symptoms. The Recognition and Prevention (RAP) program in New York is a longitudinal study prospectively following adolescents in the prodromal (i.e. pre-psychotic) phase of illness. The study is based on a neurodevelopmental framework that evaluates a combination of underlying negative and functional risk factors and the progressive emergence of positive symptoms. A long term goal is to identify risk factors beyond attenuated positive symptoms that will both contribute to an understanding of the mechanisms involved and improve predictive accuracy.

Methods: Individuals participating in the RAP program receive an extensive baseline battery consisting of behavioral, neurocognitive, clinical, and functioning measures and are followed clinically for up to five years. The study is divided into two phases, Phase I (recruitment: 2000-2006), the initial sample, and Phase II (recruitment: 2005-2012) an independent, replication sample. The data reported in this presentation includes the full Phase I sample (CHR n = 192; Healthy Controls, HC = 68), with follow-up completed in 2011. The mean age of Phase I is 16.3 years, IQ is within the normal range (mean premorbid IQ = 106; current IQ = 102) and parental SES is relatively high, indicating this to be essentially a middle class population. There is a higher percentage of males than females (not significant) and the sample is ethnically diverse. The overall sample is divided into four subgroups, depending on presence and severity of attenuated positive symptoms. The least severe of the subgroups includes individuals displaying functional deficits but no attenuated positive symptoms. The most severely ill subgroup includes individuals who meet criteria for brief intermittent psychotic symptoms, considered still at risk for fully expressed psychosis according to typical entry criteria. The two intermediate groups are characterized by attenuated positive symptoms ranging from moderate to severe but non-psychotic. Conversion rates for the 4 groups are compared using Kaplan Meier Survival Analysis. An overall predictor profile is generated by entering a comprehensive set of measures from the baseline battery using logistic regression analysis.

Results: Conversion rates peak at about 4 years and vary depending on severity of attenuated positive symptoms, with moderate positive symptoms associated with a relatively low rate (11%) and the most severe attenuated symptoms, the highest conversion rates (49%). Kaplan Meier survival analysis indicates that subgroup differences in rates based on severity at baseline is significant (log rank, $p < .001$). Survival rates also suggest that prodromal diagnoses are unstable in adolescents younger than 15. In addition, the Phase I findings have generated a risk profile improving predictive accuracy over the standard entry criteria, which incorporates specific neurocognitive deficits and social deterioration as the major non-clinical risk factors ($P < .001$; Sensitivity = 60%, Specificity = 97% and PPV = 81.8).

Conclusions: Differences in outcome as a function of baseline attenuated positive symptom severity indicate that the prodromal phase of illness is heterogeneous, supporting phase-specific interventions, with pharmacological intervention most appropriate for later prodromal phases. The mix of baseline severity levels in previous studies should be taken into consideration when interpreting the substantially broad range of conversion levels reported throughout the literature. The current findings also suggest a more extensive evaluation of current clinical entry criteria, and the need to include risk factors other than positive symptoms. The improved risk profile identified in Phase I of the RAP program indicates that two specific risk factors be added to the clinical criteria: neurocognitive deficits and increasing social difficulties.

Keywords: Prevention, Psychosis. Clinical High Risk, CHR, Prediction, Prodromal

Disclosure: B. Cornblatt, **Part 1:** Hoffman La Roche; R. Carrion, Nothing to Disclose; A. Auther, Nothing to Disclose; D. McLaughlin, Nothing to Disclose.

M137. A Randomized Trial of a Low Trapping Non-selective N-methyl-D-aspartate Channel Blocker (AZD6765) in Treatment-resistant Major Depression

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Background: The high-affinity *N*-methyl-D-aspartate (NMDA) antagonist ketamine exerts rapid antidepressant effects, but has psychotomimetic properties. AZD6765 is a low-trapping NMDA channel blocker with low rates of associated psychotomimetic effects. This study investigated whether AZD6765 could produce rapid antidepressant effects in subjects with treatment-resistant major depressive disorder (MDD).

Methods: In this double-blind, randomized, crossover, placebo-controlled study, 22 subjects with DSM-IV treatment-resistant MDD received a single infusion of either AZD6765 (150 mg) or placebo on two test days one week apart. The primary outcome measure was the Montgomery-Asberg Depression Rating Scale (MADRS), which was used to rate overall depressive symptoms at baseline; at 60, 80, 110, and 230 minutes post-infusion; and on days 1, 2, 3, and 7 post-infusion. Several secondary outcome measures were also used, including the Hamilton Depression Rating Scale (HDRS). In addition, to better understand the effects on individual depressive symptoms, the effect sizes were calculated for 17 individual HAM-D items from the present study and contrasted to the effects sizes from our previous work with MK-0657 and ketamine in patients with MDD.

Results: Within 80 minutes, MADRS scores significantly improved in subjects receiving AZD6765 compared to placebo; this improvement remained significant only through 110 minutes ($d = 0.40$). On the HDRS, a drug difference was found at 80 and 110 minutes and at day 2 ($d = 0.49$). Overall, 32% of subjects responded to AZD6765 and 15% responded to placebo at some point during the trial. No difference was observed between the groups with regard to psychotomimetic or dissociative adverse effects. HAM-D symptoms showing moderate or better ($d \geq .50$) improvement on AZD6765 were: depressed mood and psychic anxiety. Using a similar statistical model, MK-0657 appeared to moderately improve psychic anxiety and guilt, but it moderately worsened general somatic symptoms and late insomnia. However, ketamine appeared to moderately improve psychic anxiety, depressed mood, general somatic symptoms, work and interests, guilt, hypochondriasis, motor retardation, somatic anxiety, as well as early, middle, and late insomnia.

Conclusions: In patients with treatment-resistant MDD, a single intravenous dose of AZD6765, a low trapping NMDA channel blocker was associated with rapid but short-lived antidepressant effects; no psychotomimetic effects were observed. Contrasting the different NMDA antagonists studied in MDD may be useful in identifying particular symptom profile changes that occur based on their subunit selectivity and/or degree of trapping.

Keywords: antidepressant, depression, glutamate, low-trapping NMDA, rapid-acting, treatment-resistant

Disclosure: C. Zarate, **Part 1:** A patent application for the use of ketamine in depression has been submitted listing Dr. Zarate among the inventors; he has assigned his rights on the patent to the U.S. government, but will share a percentage of any royalties that may be received by the government; D. Mathews, Nothing to Disclose; N. Brutsche, Nothing to Disclose; L. Jolkovsky, Nothing to Disclose; M. Smith, **Part 1:** Full time employee with AstraZeneca Pharmaceuticals, **Part 2:** Full time employee with AstraZeneca Pharmaceuticals, **Part 3:** Full time employee with AstraZeneca Pharmaceuticals, **Part 4:** Full time employee with AstraZeneca Pharmaceuticals; D. Luckenbaugh, Nothing to Disclose.

M138. Impact of Sex and Gonadal Steroids on Neonatal Brain Structure

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Background: Relative risk for many psychiatric disorders are markedly different in males and females, but very little is known about the precise timing, neural circuitry, or mechanisms underlying these epidemiological patterns. While there are numerous reports of sexual dimorphism in brain structure in adults and older children, data on sex differences in infancy are extremely limited. This is a serious gap in knowledge which limits our ability to understand sex differences in psychiatric disorders as this is the period of most rapid postnatal cortical growth and is likely critical in disorders such autism and schizophrenia.
Methods: The primary goal of the current study was to identify sex differences in neonatal brain structure using automated region of interest volumetry and tensor-based morphometry (TBM). The secondary goal was to explore whether individual differences in brain volumes within sexually dimorphic areas were related to androgen exposure or sensitivity. 293 neonates (149 Male, 144 Female, 143 singletons, 150 twins) received structural MRI scans on a Siemens head-only 3T scanner with MP-RAGE T1-weighted, and TSE, dual-echo (proton density and T2 weighted) sequences. A genetic predisposition for high sensitivity to androgen was measured using the number of CAG triplets in the androgen receptor gene and the ratio of the 2nd to 4 digit was taken as a proxy measure of prenatal androgen exposure. For automated ROI volumetry, mixed models were used to study the relationship between brain tissue volumes, sex, CAG repeat length, and digit ratios. For TBM, cluster-based inference was used to identify relationships between local gray matter volume, sex, CAG repeat length, and digit ratios.
Results: Automated ROI volumetry showed a significant sex difference of 5.87% for intracranial volume ($p < 0.0001$), but there were no significant sex differences in lobar tissue volumes after adjusting for ICV. ICV was not related to CAG repeat length or digit ratios. In contrast, TBM identified extensive areas of local sexual dimorphism. In particular, males had larger volumes in medial temporal cortex and rolandic operculum and females had larger volumes in dorsolateral prefrontal cortex, motor cortex, and visual cortex. Androgen exposure and sensitivity had some minor effects on local gray matter volume, but did not appear to be the primary determinant of sexual dimorphism in this age group.
Conclusions: Comparing our study to similar studies in older children and adults suggests that sex differences in cortical structure vary in a complex and highly dynamic way across the human lifespan. Results can be grouped into 4 general patterns. (1) Sex differences which are stable across the lifespan. (2) Sex differences which are unique to the neonate. (3) Sex differences which are present during periods of high circulating gonadal steroids (e.g. neonate and young adult, but not childhood). (4) Sex differences which are not present in the neonate but arise during childhood and/or adolescence. These divergent spatiotemporal patterns suggest that multiple biological mechanisms contribute to sexual differentiation of the brain. In particular, the relative lack of androgen effects in the neonate suggests that direct sex chromosome effects may be of primary importance in this age group. This is the first study to provide detailed information on sex differences in global, regional, and local brain volumes in the neonate. As such, replication in independent samples is critical. Additional studies are also needed to test whether these structural differences have functional consequences for cognitive development and psychiatric risk.
Keywords: Sex difference, gender, testosterone, morphometry

Disclosure: R. Knickmeyer, **Part 4:** RK is co-investigator (no salary support) on 2 grants from Pfizer; J. Wang, Nothing to Disclose; H. Zhu, Nothing to Disclose; X. Geng, Nothing to Disclose; S. Woolson, Nothing to Disclose; R. Hamer, **Part 1:** RH has served on a DSMB, an advisory board, consulted or advised, or is involved in a contract between UNC and one of the following institutions: Abbott, Acadia, Allergan, Alkermex, Alpharma, AstraZeneca, Cenerx, Columbia U, Corcept, Endo, Eli Lilly, EnabledMD, Epix, J & J, NeuroPharmaBoost, Novartis, Pepper-Hamilton, Pfizer, PureTechVentures, Roche (Genentech), SAS, Schwartz, Solvey, Sanofi-Aventis, Takeda, Winston & Strawn, Wyeth, and NeurogensX. RH and/or his wife own stock in Bristol-Myers Squibb, Amgen, Lilly, Genentech, Proctor and Gamble, and Sepracor. RH's wife is retired from and has stock options in Bristol-Myers Squibb; T. Konneker, Nothing to Disclose; M. Styner, Nothing to Disclose; J. Gilmore, **Part 4:** JG previously had a research grant with Dainippon Sumitomo Pharma.

M139. Stress Response Systems in Adolescent Girls and Boys with Major Depression: A Multi-Modal Approach

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Background: The pathophysiology of major depressive disorder (MDD) involves impairment within the neurobiological systems that underlie the response to stress. The neuroendocrine stress response system, encompassed by the Hypothalamic Pituitary Adrenal (HPA), is centrally implicated in MDD. Additionally, fronto-limbic neural circuitry is (a) implicated in MDD, (b) associated with stress response and (c) tightly linked with the HPA system. Core components of this network include the amygdala and the rostral anterior cingulate cortex (rACC). Sex differences have previously been identified in brain development and in stress response. However, the functioning of neurobiological stress systems in adolescents has been understudied. This research is particularly important in adolescence as neurobiological systems are still undergoing development. The goal of the present study was to examine the neurobiological stress systems in adolescent girls and boys with MDD.
Methods: Participants included 54 adolescents aged 12-19, including 39 with MDD (22 unmedicated, 17 medicated) and 15 healthy comparison volunteers. All participants underwent diagnostic evaluation, the Trier Social Stress Test (TSST), and brain imaging (which included a T1 anatomical scan). Statistical analyses were conducted using SPSS. In the TSST, participants were asked to prepare and deliver a short speech to a strange audience. Salivary cortisol measurements were taken at the beginning and immediately following the procedure, and at 15 minutes intervals afterward for a total of five time points. Repeated measures analysis was conducted on the cortisol levels, including group (control, medicated depressed and unmedicated depressed) and sex as fixed effects. Multivariate regression analyses were also conducted on peak cortisol levels and area under the curve measurements to assess for effects of group and sex. Analysis of anatomical imaging data was conducted using the FreeSurfer software to extract brain volumes from key regions of interest. A multivariate regression analysis was conducted on key fronto-limbic brain regions that were hypothesized to relate to MDD and the stress system: bilateral amygdala volumes and bilateral rACC volumes, including group and sex as fixed effects, and intracranial volume as a covariate. Finally, we examined correlations between cortisol measurements (peak levels and area under the curve) and brain volumes (amygdala and rostral anterior cingulate.)
Results: For the TSST, repeated measures analysis revealed a significant group by time effect ($F = 3.4$, $p = 0.002$) and a trend-level sex effect ($F = 2.0$, $p = 0.1$). The control group exhibited a

normative elevation in cortisol followed by return to baseline; the unmedicated group showed elevated baseline cortisol levels, peaked higher and remained elevated at the end of the experiment; and the medicated group showed a marked decrease during the task followed by a return to baseline. Examination of the results separately by sexes showed that while depressed boys tended to show an over-responding pattern, the depressed girls showed an under-responding pattern. Medication seemed to flatten responses for both boys and girls. Multivariate analyses revealed of peak and summary cortisol measures showed significant effects for group ($F = 3.7$, $p = 0.000$), sex ($F = 2.6$, $p = 0.03$), and a group by sex interaction ($F = 2.8$, $p = 0.003$). Whereas unmedicated boys had much higher peak and summary levels than both controls and medicated boys, the female groups were more similar. Finally, although we did not identify significant group effects with respect to brain volumes for our regions of interest, we did find significant correlations between both left and right amygdala volume and peak cortisol during the recovery phase of the experiment (left: $r = -0.4$, $p = 0.006$; right: $r = -0.3$, $p = 0.03$) as well as with the summary measure (area under the curve) (left: $r = -0.4$, $p = 0.008$, right: $r = -0.3$, $p = 0.03$.)

Conclusions: We report results from a multi-method study that examined stress systems in adolescents with MDD. Our findings suggest that the systems that underlie the stress response in adolescents with depression are abnormal, with unmedicated adolescents showing elevated stress responses and delayed recovery. Current treatment with medication appears to dampen the biological response to social stress. These cross sectional results suggesting the impact of treatment on HPA responding should be followed by longitudinal studies to directly test whether treatment mitigates the stress response in depressed teens. Although this study included fewer boys than girls, tentatively our results suggest that unmedicated depressed boys show an over-responding pattern, whereas for girls the pattern is that of under-responding. The sex effects noted in this study require confirmation with larger and more balanced samples. Finally, although fronto-limbic brain volumes did not differentiate groups, they were inversely related to cortisol measures in the adolescents of this study. Additional research using multi-modal approaches is needed to further delineate the inter-dependent relationships across neurobiological stress systems.

Keywords: depression adolescents cortisol amygdala

Disclosure: K. Cullen, Nothing to Disclose; B. Klimes-Dougan, Nothing to Disclose; A. Houri, Nothing to Disclose; K. Lim, Nothing to Disclose.

M140. Stress Response in Adolescents with Childhood Maltreatment: Moderation by Gender and Genetic Factors

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Background: The developing brain is highly sensitive to the effects of early-life stress and maltreatment (MALTX), and early-life adversity has been associated with alterations in the stress response systems. However, there is heterogeneity in neurobiological alterations and emotional/behavioral outcomes. Little information is available on the factors moderating neurobiological alterations. The purpose of this research was to compare hypothalamic-pituitary-adrenal (HPA) response to a standard psychosocial stressor in adolescents with and without childhood MALTX history. To examine whether single-nucleotide polymorphisms (SNPs) of the corticotropin-releasing hormone (CRH) receptor gene will moderate the HPA response.

Methods: Seventy-three adolescents (31 males, 42 females) with MALTX and 35 adolescent controls (15 males, 20 females) without MALTX were administered a psychosocial stressor (modified Trier Social Stress Test). Salivary cortisol samples were collected before and after the stressor. CRHR1 SNPs were genotyped using the *Illumina GoldenGate Genotyping Assay*.

Results: There was an interaction between MALTX status and gender on HPA response. Consistent with prior reports, control males showed higher HPA reactivity than control females. MALTX females had a delayed and more exaggerated HPA reactivity than their counterparts without MALTX. This pattern was reversed in males. MALTX also interacted with CRHR1 SNPs (rs110402 and rs242924) to modulate HPA response to the stressor. Genotypes associated with CRHR1 SNPS did not significantly influence HPA reactivity among controls. However, MALTX youth that were AA-homozygous had a blunted response and those individuals that were GG-homozygous had a highly robust HPA response (rs110402). A similar effect was seen for the rs242924. MALTX GG –homozygotes and higher HPA reactivity participants showed more severe self-reported depressive symptoms.

Conclusions: The moderating effects of gender and CRHR1 SNPs on the relationship between HPA reactivity and MALTX suggests a differential risk for emotional (high HPA reactivity) and behavioral (blunted HPA response) problems. Longitudinal data are available on this cohort. These data will be analyzed to examine the interactions among MALTX, gender and genetic SNPs in the differential longitudinal clinical course (i.e., development of emotional disorders, behavioral disorders, and their comorbidity).

Keywords: adolescent, HPA axis, genetic, stress, maltreatment

Disclosure: U. Rao, Nothing to Disclose; E. Gorodetsky, Nothing to Disclose; D. Goldman, Nothing to Disclose.

M141. Salivary Cortisol, Pro-Inflammatory Cytokines, and Grief-Related Psychiatric Symptoms in Parentally Bereaved Children

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Background: Although parental loss in childhood is a serious public health issue, the field of childhood grief has continued to remain in its infancy over the last decade, especially in terms of differentiating "normal" versus "maladaptive" grief. The imminent arrival of DSM-V and its introduction of a new bereavement-related disorder, Persistent Complex Bereavement Disorder, makes this an auspicious time to conduct studies that help shed light on potential biological and psychosocial mechanisms linking childhood bereavement to grief-related psychopathology. The hypothalamic-pituitary adrenal (HPA) axis and its end product, cortisol, play a key role in mediating the negative impact of stress on the pathophysiology of psychiatric disorders and modulation of stress-induced inflammatory states. A pro-inflammatory cytokine, IL-1β, provides further feedback regulation to the hypothalamus, increasing HPA cortisol output to protect against an unabated rise in pro-inflammatory cytokines. Emerging evidence identifies chronic post-traumatic stress symptoms to be positively associated with (1) dampening of the cortisol awakening response, (2) inflammatory hypersensitivity to cortisol, and (3) increased somatic complaints in adults. Recent work in our own lab has demonstrated links between a dampening of the cortisol awakening response and posttraumatic stress symptoms in bereaved youth. In addition, contrary to the adult literature, we found that youth who experienced the anticipated death of a loved one exhibit *higher* levels of posttraumatic stress symptoms compared to those who experienced a sudden natural death. However, no studies to date have simultaneously investigated potential relations between pro-inflammatory cytokines, salivary cortisol, circumstances of the death, and grief-related psychiatric symptoms in bereaved youth. Thus, the goal of the current pilot study was to examine associations between biological (salivary cortisol, salivary IL-1β), psychological (posttraumatic stress, somatization) and environmental (circumstances of death) factors in a sample of recently parentally bereaved children.

Methods: Participants included 19 parentally bereaved children between the ages of 3 and 12 years old who lost a parent within the previous six months. All participants were administered a semi-structured interview focused on their thoughts and feelings related to the death. Participating children between the ages of 7 and 12 were also administered a battery of psychological and behavioral assessments. Measures of relevance to the current study include (1) the UCLA PTSD Reaction Index, a child-self report measure of children's own posttraumatic stress symptoms; and (2) the Child Behavior Checklist for Children, a parent-self report measure of the child's internalizing and externalizing symptoms, including somatization. Cause of death was coded from parents' reports and the sample was divided between those who experienced an anticipated death and those who experienced a sudden natural or accidental death. Participants were asked to provide saliva samples in their homes at three different time points (upon awakening, 30 minutes later, and in the evening) over the course of 3 days, beginning the day after the interview. Saliva was collected using Salivette sampling tubes and samples were frozen until thawed, centrifuged, and the supernatants assayed for both cortisol and IL-1β using standard ELISA techniques following manufacturer instructions (R&D Systems, MN). Area under the curve (AUC) was calculated for both salivary cortisol and IL-1β.
Results: Student's T-test was used to compare both cortisol and IL-1β across the two cause of death groups and Spearman's correlation tested associations between cortisol, IL-1β and symptoms of post-traumatic stress and somatization. Salivary $cortisol_{AUC}$ was negatively correlated with posttraumatic stress symptoms ($rho = -0.59$, $p = 0.026$, $n = 19$). Participants who anticipated parental death had reduced salivary $cortisol_{AUC}$ ($T_{19} = -2.4$, $p = 0.02$) and increased PTSD symptoms ($T_9 = 2.7$, $p = 0.02$) as compared to those who experienced a sudden death. Baseline Salivary IL-1β was positively correlated with a measure of somatic problems (subscale of the Child Behavior Checklist) ($r = 0.54$, $p = 0.05$, $n = 13$). In contrast, salivary $IL\text{-}1\beta_{AUC}$ was marginally, but negatively correlated with posttraumatic stress symptoms ($rho = -0.59$, $p = 0.09$, $n = 9$). On visualization of the graphs of IL-1β, trend elevation in salivary IL-1β was evident in those children who had anticipated the death of parent versus those who experienced a sudden death (statistical testing for significance not possible due to missing data).
Conclusions: Findings suggest that bereaved children who experience the anticipated death of a parent (compared to a sudden death) may show dampening of salivary $cortisol_{AUC}$ and a possible elevation (i.e. less reduction) in salivary IL-1β, along with increased posttraumatic stress symptoms. Ongoing data collection will allow us to determine whether these results are indicative of chronic stress predisposing children to inflammatory hypersensitivity to cortisol, particularly in the context of posttraumatic stress. Preliminary findings speak to the importance of understanding the circumstances surrounding the death itself and may shed light on potential biological mechanisms associated with vulnerability to posttraumatic stress in parentally bereaved youth.
Keywords: Cortisol, cytokines, grief, children, posttraumatic stress
Disclosure: J. Kaplow, Nothing to Disclose; K. Wiese, Nothing to Disclose; J. Abelson, Nothing to Disclose; A. Prossin, Nothing to Disclose.

M142. Circadian Cortisol and Hypothalamic-Subgenual Cortex Functional Connectivity in Major Depression with and without Psychotic Features

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Background: The hypothalamus drives the hypothalamic-pituitary-adrenal axis hormone cascade and increases cortisol release. The circadian rhythm of cortisol is entrained to the photoperiod and regulated by central inhibitory feedback mechanisms. The hypothalamus also coordinates emotional behaviors, reproductive drive, and other appetitive behaviors through direct neural connections with the rest of the brain. Disturbances in the secretion and regulation of cortisol have been linked closely with adverse impacts on these other hypothalamic functions. In the current study we test for relationships amongst 1) circadian cortisol secretion disturbances, 2) abnormal hypothalamic functional connectivity with subgenual cortex 3) and specific subtypes of major depression.
Methods: In a total of 102 subjects consisting of patients with major depression without psychotic features (NPMD), major depression with psychotic features (PMD), and healthy controls (HC) we collected circadian cortisol measures, resting state functional brain activity, and symptom data. Overnight total cortisol was measured in blood every hour from 6 p.m. to 9 a.m. Resting-state functional brain activity was collected in a 5-minute functional scan in which participants rested with their eyes open. Salivary cortisol was collected immediately before and after the scan. Depressive and psychotic features were assessed using the Hamilton Depression Rating Scale (HAMD), the Brief Psychiatric Rating Scale (BPRS), and the Structured Clinical Interview for DSM-IV. To insure that NPMD's were of similar depressive severity to PMD subjects, all depressed patients were required to have a minimum score on the HAMD Thase Endogenomorphic Subscale indicative of clinically significant endogenous symptoms.
Results: Patients with major depression with psychotic features demonstrated significantly elevated cortisol levels between 6 pm and 1 am compared to healthy participants. This pattern was not evident in patients without psychotic features. Healthy participants had strong connectivity between the hypothalamus seed region and the subgenual cortex. Patients with psychotic features had significantly reduced functional connectivity between these regions. Both patient groups had a qualitatively similar pattern of reduced hypothalamus-subgenual cortex connectivity, but only the patients with psychotic features achieved statistical significance relative to healthy participants. Within patients with psychotic features, functional connectivity between the hypothalamus and the subgenual cortex was negatively correlated with cortisol levels in the evening phase (Pearson's $r = -.394$, $p < 0.05$). This relationship was not observed in either of the other participant groups, or within the pooled groups.
Conclusions: These results show for the first time that the hypothalamus has strong resting state functional connectivity with the subgenual cortex. They also suggest that for subjects with major depression with psychotic features, there is a relationship between their decreased functional connectivity and their cortisol hypersecretion during the evening hours of the circadian rhythm. The directionality of this relationship however is unclear and bidirectional effects are plausible. Since the circadian rhythm is driven by output from the hypothalamus, subgenual inputs could be modulating this drive. In addition, both the subgenual cortex and the hypothalamus contain corticosteroid receptors and therefore are susceptible to cortisol impacting the strength of connectivity between these regions.
Keywords: Cortisol, Depression, Hypothalamus, Psychosis, Imaging
Disclosure: K. Sudheimer, Nothing to Disclose; J. Keller, **Part 1:** Stanford University, Palo Alto University, **Part 2:** Stanford University, Palo Alto University, **Part 3:** Stanford University, Palo Alto University, **Part 4:** None.; L. Tennakoon, Nothing to Disclose; A. Schatzberg, **Part 1:** Stanford University, American Psychiatric Association, PharmaNeuroBoost, Lilly, Cervel, Neurocrine, X-Hale, Pfizer, Corcept, Merck, Forest labs, **Part 2:** Stanford University, Corcept Therapeutics, PharmaNeutroBoost, Pfizer, Forest Labs, Neurocrine, American Psychiatric Association, **Part 3:** Pharma-NeuroBoost, Stanford University.

M143. Blood Glucagon-Like Peptide-1 (GLP-1) Concentration Correlates Inversely with Alcohol Self-Administration in a Laboratory Study with Alcoholic Individuals: Preliminary Findings

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Background: There is robust evidence that food- and alcohol-seeking behaviors share the same neurobiological pathways, and that feeding-related peptides may play a role in alcohol-seeking behavior. Previous studies have focused on the role of anorexigenic hormones such as leptin and insulin in alcoholism, but no clinical studies have been conducted testing the possible role of Glucagon-Like Peptide-1 (GLP-1). The gut peptide anorexigenic hormone GLP-1 produces several physiological effects in the periphery including control of glucose homeostasis; additionally, GLP-1decreases food intake by increasing satiety in brain. The anorexigenic properties of GLP-1 and its central effects lead to suggest that GLP-1 might potentially represent an interesting peptide to investigate in alcoholic individuals.
Methods: Blood GLP-1 levels were analyzed in a small group of subjects who took part into a human laboratory pilot study where participants performed a cue-reactivity and an alcohol self-administration procedure. While the main goal of this pilot study was to investigate the role of baclofen, as compared to placebo, on alcohol-related outcomes in 13 non-treatment seeking alcoholic individuals, the analysis presented here was conducted after controlling for the medication condition. The goal of this analysis was to investigate the possible correlation between blood GLP-1 levels and alcohol self-administration expressed as the number of standard drinking units (SDUs) consumed in a bar-like laboratory room.
Results: The analysis of blood GLP-1 concentration collected before starting the ASA (e.g. when breath alcohol concentration was zero) relieved a statistically significant negative correlation between GLP-1 concentrations an alcohol consumed during the ASA ($r = -.83$; $p = .02$). We also analyzed additional anorexigenic hormones, and with the exception of PYY, all other hormones (leptin, insulin, GIP, PP) were negatively correlated to the amount of alcohol consumed during the ASA; unlike GLP-1, however, none of them reached statistical significance.
Conclusions: This study provides preliminary evidence that blood GLP-1 concentration correlates significantly and negatively with alcohol drinking, thus suggesting an inverse relationship between GLP-1 and alcohol drinking in alcoholic individuals. GLP-1 receptors (GLP-1Rs) are expressed in several key nodes of the mesolimbic reward system, including the ventral tegmental area (VTA) and a recent preclinical study reported that activation of central GLP-1Rs suppresses food reward/motivation by interacting with the mesolimbic system. Similarly, GLP-1R stimulation may suppress alcohol reward, thus resulting in a reduced motivation to consume alcohol. Consistent with this hypothesis, the preliminary clinical findings here reported suggest that higher blood GLP-1 concentrations are significantly related to lower alcohol consumption. As such, drugs manipulating the GLP-1 signaling might represent a novel pharmacological approach to treat alcoholism. Notably, in this study both blood GLP-1 levels and alcohol consumption were assessed under well-controlled conditions in a human lab setting. A significant limitation of this study, however, was the small sample size, thus future research is needed to investigate these preliminary findings.
Keywords: alcoholism, GLP-1, alcohol self-administration
Disclosure: L. Leggio, **Part 1:** Advisor for D&A Pharma and CT Sanremo; W. Zywiak, Nothing to Disclose; S. Edwards, Nothing to Disclose; S. Fricchione, Nothing to Disclose; Vuittonet, Nothing to Disclose; R. Swift, **Part 1:** Advisor for D&A Pharma and CT Sanremo; G. Kenna, **Part 1:** Advisor for CT Sanremo.

M144. Neuroadaptive Changes after Chronic Lurasidone Treatment: Implication for Mood and Stress-related Disorders

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Background: It is well accepted that prolonged treatment with psychotropic drugs determines a variety of neuroadaptive changes, which are supposed to normalize defective genes and proteins associated with mental illness and may promote the expression of systems that contribute to functional recovery. In particular it is know that neuroplastic proteins, such as the neurotrophin brain derived neurotrophic factor (BDNF), play a crucial role in these adaptive mechanisms. The modulation of these systems in key brain regions represents an important functional readout for the amelioration of stress-related disorders and cognitive deterioration. The characterization of these adaptive mechanisms after chronic exposure to different psychotropic drugs is important in order to establish receptor and synaptic mechanisms that may prove more effective in boosting neuronal plasticity. In the present study we investigated the neuroplastic properties of lurasidone, a novel antipsychotic drug whose dopamine D2 receptor antagonism is associated with high affinity for serotonin 5HT7 and 5HT2a receptors, moderate antagonistic activity at alpha2C- adrenergic receptors and partial agonist activity at 5-HT1A receptors.
Methods: The neuroadaptive changes set in motion by chronic treatment with lurasidone were investigated in normal rats, using different drug doses, as well as in animals models of stress-related disorders, including serotonin transporter knockout (SERT KO) rats, a genetic model characterized by an anxious and depressive, as well as rats exposed to stress during the last week of gestation (PS), a model that recapitulates the long-lasting consequences of early life adversities. Animals were treated with lurasidone (dose range 1 to 10 mg/kg/day) for 2 or 3 weeks. Treatments were performed in adulthood (normal rats or SERT KO rats) or during adolescence (PS rats). Real time PCR was used for gene expression analyses, whereas western blot was employed for protein analyses.
Results: We found that the expression of BDNF in normal animals is significantly modulated by lurasidone in a dose- and brain region related manner. Indeed the neurotrophin expression is significantly up-regulated in the hippocampus by lurasidone already at lower doses, suggesting that receptor targets toward which lurasidone shows high affinity, such as serotonin 5-HT7 receptors, may be relevant for such modifications. The modulation of BDNF in prefrontal cortex is primarily occurring at higher doses of lurasidone and may be restricted to selective populations of neurotrophin transcripts. Interestingly chronic treatment with lurasidone at 10 mg/kg is highly effective in normalizing reduced expression of BDNF in SERT KO rats, with significant improvement of fear extinction. Moreover chronic treatment of SERT KO rats with lurasidone is associated with restorative changes in the expression of several GABAergic markers, whose expression is also altered in this model of stress-related disorders. Lastly, we found that chronic lurasidone treatment during adolescence can increase BDNF expression in prefrontal cortex and may prevent the reduction of neurotrophin expression set in motion by prenatal stress exposure.
Conclusions: Our results demonstrate that lurasidone, characterized by a signature receptor profile, has the ability to modulate the expression of the neurotrophin BDNF and to normalize its defects associated with genetic or environmental animal models of stress-related disorders. The adaptive changes set in motion by repeated treatment with lurasidone may contribute to the amelioration of functional capacities, closely associated with neuronal plasticity, which are deteriorated in patients with schizophrenia, bipolar disease and major depression.
Keywords: BDNF, stress, GABA, Animal models

Disclosure: M. Riva, Nothing to Disclose; A. Luoni, Nothing to Disclose; F. Calabrese, Nothing to Disclose; G. Guidotti, Nothing to Disclose; G. Racagni, **Part 1:** I received honoraria as lecturer or speaker from Eli Lilly, Servier and InnovaPharma, **Part 4:** I received research grants from Eli Lilly and Servier; J. Homberg, Nothing to Disclose.

M145. Substrate-Selective Inhibition of Cox-2 as a Novel Strategy for *in Vivo* Endocannabinoid Augmentation

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Background: Over the past two decades the known roles of endogenous cannabinoids in the regulation of physiological and pathophysiological processes have grown exponentially. The two primary endocannabinoid ligands anandamide and 2-arachidonoylglycerol (2-AG) can activate cannabinoid receptors, CB1 and CB2. Importantly, pharmacological strategies that augment endocannabinoid signaling have been suggested to be viable treatments for mood and anxiety disorders. To date, inhibition of the anandamide degrading enzyme fatty-acid amice hydrolase (FAAH) and the 2-AG degrading enzyme monoacylglycerol lipase (MAGL) have been most extensively validated in preclinical studies. In addition to FAAH and MAGL, *in vitro* studies suggest that cyclooxygenase-2 (COX-2) can degrade both anandamide and 2-AG, however the role of COX-2 in the physiological regulation of endocannabinoid signaling *in vivo* has not been demonstrated to date. Moreover, all current inhibitors of COX-2 have profound effects on prostaglandin synthesis *in vivo*, which contributes to the cardio/cerebro-vascular adverse effects of COX-2 inhibitors, thus limiting pharmacological approaches to modulate endocannabinoid signaling via COX-2 inhibition.
Methods: We developed and screened novel substrate-selective inhibitors of COX-2 *in vitro* using purified human COX-2. Compounds that demonstrated inhibition of COX-2 activity only when the endocannabinoids anandamide and 2-AG were used as substrates, but not when arachidonic acid is used, were developed and optimized. Here we describe the *in vivo* effects of LM-4131, our most selective and potent inhibitor. We used LC/MS/MS to determine the effects of LM-4131 on brain endocannabinoids, related-lipids, and prostaglandins in the brain, liver and lung. We used behavioral assays of anxiety to determine the preclinical efficacy of LM-4131 for anxiety disorders.
Results: Our data show that COX-2 regulates central endocannabinoid signaling under physiological conditions *in vivo*. Specifically, inhibition of COX-2 increased brain AEA levels in wild-type, but not COX-2 knock out mice. We show that substrate-selective pharmacological inhibition of COX-2 can selectively increase brain endocannabinoids with a high degree of specificity over related non-cannabinoid lipids, without affecting central or peripheral prostaglandin synthesis. We also demonstrate the the biophysical determinants of substrate-selective pharmacology using structural studies and site-directed mutagenesis of purified COX-2. Lastly, we show that substrate-selective inhibition of COX-2 exerts anti-anxiety effects via activation of endocannabinoid signaling and CB1 cannabinoid receptors. Moreover, substrate-selective inhibition of COX-2 does not cause gastric bleeding or overt cannbis-like behavioral effects in mice suggesting a low propensity for GI and psychotomimetic side-effects of LM-4131. These findings solidify COX-2 as a major *in vivo* regulator of endocannabinoid signaling and validate substrate-selective pharmacology as a viable approach to selectively enhance endocannabinoid signaling for therapeutic gain.
Conclusions: Overall, our findings provide a new conceptualization of central endocannabinoid metabolism to prominently include COX-2, in addition to FAAH and MAGL, and provide a viable pharmacological strategy to capitalize on the therapeutic potential of COX-2 regulation of endocannabinoid signaling. That endocannabinoid signaling is involved in broad range of pathological and physiological processes, and the widespread inducible expression of COX-2, our findings could have far reaching applicability to fields including metabolic regulation, neuro-inflammation, as well as other neuropsychiatric disorders.
Keywords: cannabinoid, COX-2, anxiety, anandamide, prostaglandin
Disclosure: D. Hermanson; N. Hartley, Nothing to Disclose; L. Marnett, **Part 2:** Vanderbilt University has a patent application pending for substrate-selective inhibitors of COX-2 inlcuding LM-4131. No income has ben generated form this application; S. Patel, **Part 2:** Vanderbilt University has a patent application pending for substrate-selective inhibitors of COX-2 inlcuding LM-4131. No income has ben generated form this application.

M146. Modulation of the Inflammatory Response after the Antidepressant Agomelatine in Rats

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Background: Major depression is a severe psychiatric disorder that is the fourth leading cause of disability in the world accounting for a relevant percentage of morbidity. One of the major problems associated with depression is the relevant percentage of patients who do not show an adequate response to antidepressant therapy, as well as the high rate of relapse. For this reason, there has always been a great deal of interest in understanding the molecular mechanisms contributing to depression etiopathology as well as in identifying systems and pathways that may play a critical role in antidepressant response. Beside the well-established involvement of systems such as neurotransmitters, hormones and neuroplasticity mediators, compelling evidences indicate that altered inflammatory activity is associated with depression. Specifically, increased concentration of pro-inflammatory cytokines has been found in the blood and also in the cerebrospinal fluid of depressed patients. Moreover, cytokine administration induces depressive symptoms, as occurs in the 30% of hepatitis C patients who are treated with the immune activator interferon-alpha. Finally, depression shows elevated co-morbidity with pathologies associated with peripheral activation of inflammatory cytokines such as cardiovascular disease, cancer, arthritis rheumatoid and neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Based on these observations, aim of this study was to evaluate the ability of the novel antidepressant agomelatine to modulate specific components of the immune response in the rat hippocampus following a systemic inflammatory challenge with the cytokine-inducer lipopolysaccharide (LPS).
Methods: Adult male rats received agomelatine (40 mg/kg, p.o.) or vehicle for 21 days before being challenged with an acute injection of LPS (250 mg/kg; i.p.) 16 h after the last drug administration. Rats were sacrificed 2, 6, or 24 h after the inflammatory challenge, in order to establish the ability of agomelatine to interfere with the initial (2-6 h) or the later phase (24 h) of the inflammatory response. Real time PCR was used for gene expression analyses at central levels whereas ELISA was employed for protein analyses at plasma level.
Results: We first evaluated the inflammatory response by measuring the mRNA levels of different pro-inflammatory cytokines i.e. Interleukin-1b (IL-1b) and Interleukin-6 (IL-6) in different rat brain regions (ventral and dorsal hippocampus and prefrontal cortex). We found that acute LPS injection markedly increased the expression of these cytokines in vehicle-treated rats with a specific temporal profile. Interestingly, pre-treatment with agomelatine significantly reduced the LPS-induced up-regulation

of IL-1b and IL-6, an effect also observed at peripheral level, where agomelatine completely blocked the increased cytokine plasma levels observed 6 h after the inflammatory challenge. At central level, these effects appear to be associated to inhibition of NF-kB-driven transcription as well as alteration of mechanisms responsible for microglia activation. Specifically, the antidepressant limited the LPS-induced up-regulation of CD11b, one of the most used markers for this cellular phenotype, with a parallel increase of CD68, suggesting the induction of active phagocytosis that might contribute to the anti-inflammatory effect of agomelatine. The attenuation of microglia activation by agomelatine involves neuron-glia cross-talk. Indeed, LPS reduced the expression of neuronal CX3CL1 thus leading to increased microglia activation, an effect normalized by the antidepressant. In addition, we found that agomelatine was also able to alter basal and LPS-induced changes of the kynurenine pathway, which represents a point of convergence between inflammatory and neurotransmitter defects associated with depression.

Conclusions: Taken together, these data disclose novel properties that may contribute to the therapeutic effect of agomelatine, providing evidence for a crucial role of specific components of the immune/inflammatory system in the antidepressant response and thereby in depression etiopathology and in the treatment of depressed patients with organic diseases co-morbidity.

Keywords: Agomelatine, Antidepressant drug, Inflammatory response, Mood disorders

Disclosure: R. Molteni, Nothing to Disclose; F. Macchi, Nothing to Disclose; C. Zecchillo, Nothing to Disclose; M. Dell'Agli, Nothing to Disclose; M. Riva, Nothing to Disclose; G. Racagni, Nothing to Disclose.

M147. Synergy between Melatonergic and 5-HT2C Receptors in the Action of Agomelatine – Molecular and Cellular Evidence

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Background: Agomelatine is an innovative antidepressant licensed by the European Medicines Agency for the treatment of major depression. The primary mechanism of action of agomelatine is related to its action as an agonist of melatonergic 1 (MT1) and 2 (MT2) receptors as well as an antagonist for serotonergic 5-HT_{2C} receptors. Agomelatine has proven antidepressant efficacy in both preclinical and clinical studies. Aim of this work was to dissect the involvement of MT1/MT2 agonism and 5-HT_{2C} antagonism in the effect of agomelatine by studing the following variables: 1) the expression of the neurotrophin Brain-Derived Neurotrophic Factor (BDNF) and the immediate early gene Activity-regulated cytoskeleton-associated protein (Arc), two functionally related genes involved in neuroplasticity and antidepressant mechanism of action; 2) the depolarization-evoked glutamate release from synaptosomes of Prefrontal and Frontal Cortex (P/FC) induced by acute footshock (FS)-stress.

Methods: Study 1) Adult male rats were acutely treated with agomelatine (40 mg/kg, i.p.) or vehicle (HEC1%, 1 ml/Kg i.p.) and killed 1 h, 7 h or 16 h after drug administration (17.00 pm) in order to investigate the time course effect of agomelatine on BDNF and Arc expression. In order to establish the contribution of MT1/2 and 5-HT_{2C} receptors rats were acutely treated with agomelatine (40 mg/kg, i.p.), melatonin (40 mg/kg i.p.), the 5-HT_{2C} selective antagonist S32006 (10 mg/kg i.p.) or vehicle. Total RNA was isolated from prefrontal and frontal cortex. BDNF and Arc mRNA levels were assessed by Real Time PCR. Results were analyzed by two-way ANOVA with SCPHT and one-way ANOVA with Fisher's PLSD (as appropriate). Study 2) Adult male rats were treated for 14 days with agomelatine (40 mg/Kg i.p.), melatonin (40 mg/Kg i.p.), S32006 (10 mg/Kg i.p.) or vehicle (HEC 1%, 1 ml/Kg i.p.). Treatments were performed 2 h before dark (17:00 pm), as in study 1. 16 h after the last administration, rats were subjected to acute Foot Shock-stress (FS). Prefrontal/Frontal cortex (P/FC) was immediately dissected and synaptosomes were prepared by differential centrifugation on Percoll gradients. Glutamate and GABA release was measured in freshly purified synaptosomes by using superfusion technique. Results were analyzed by one-way ANOVA with post-hoc Newman Keuls test.

Results: Study 1) We found that BDNF mRNA expression was significantly lower in animals sacrificed in the morning than in the evening. Acute agomelatine treatment prevents the decrease of BDNF: 16 h after injection, BDNF mRNA levels were higher in agomelatine-treated rats than in vehicle-treated rats. Acute melatonin or S32006 alone did not produce any significant change in BDNF expression. Agomelatine significantly elevated Arc mRNA levels at the different time points. Neither melatonin nor S32006 alone was able to mimic agomelatine effect on Arc expression. Study 2) Acute FS stress increased depolarization-dependent release of endogenous glutamate from P/FC synaptosomes and chronic agomelatine treatment completely prevented the stress-induced increase of glutamate release. However, melatonin or S32006 alone were not able to reproduce agomelatine effects, although a trend for reduction of stress-induced glutamate release was observed with S32006. No significant changes in GABA release were induced by either FS-stress or any of the drugs.

Conclusions: In summary our data demonstrate that: 1) an acute injection of agomelatine modulates the expression of neuroplastic genes (BDNF and Arc) in selected brain structures 2) chronic treatment with agomelatine significantly prevented the stress-induced increase of glutamate release from P/FC synaptosomes. The present results, showing that neither melatonin nor a selective 5HT_{2C} receptor antagonist mimic agomelatine effects, strongly suggest that agomelatine effects at the cellular level result from a synergistic interaction between its action on MT1/MT2 and 5-HT_{2C} receptors. This interaction underlies the efficacy of agomelatine in terms of restoring circadian rhythms and relieving depressive symptoms.

Keywords: agomelatine, glutamate, BDNF, ARC, synergism

Disclosure: D. Tardito, Nothing to Disclose; M. Riva, Nothing to Disclose; R. Molteni, Nothing to Disclose; A. Mallei, Nothing to Disclose; L. Musazzi, Nothing to Disclose; F. Calabrese, Nothing to Disclose; M. Popoli, Nothing to Disclose; G. Racagni, Nothing to Disclose.

M148. Spatial Memory Deficits in Adult c57BL6 Mice Exposed to Fluoxetine during Adolescence

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Background: Epidemiological reports indicate that major depressive disorders (MDD) are common in children and adolescents. The prevalence of adolescent MDD has resulted in parallel increases in the prescription of fluoxetine (FLX) and related serotonin reuptake inhibitor antidepressants within this population. Although such treatments can last for years, very little is known about the long-term consequences of antidepressant exposure during developmental periods prior to adulthood on spatial memory performance later in life.

Methods: Adolescent male c57BL/6 mice (postnatal day [PD] 35) were exposed to FLX (10 mg/kg, twice daily) for 15 consecutive days. We then assessed animals' behavioral performance on the Morris water maze spatial memory task when they reached adulthood (PD 70 +). Specifically, mice were trained to find the location of a submerged escape platform on a single day task (8 training trials), and memory for the platform location was

re-tested after a 24 hr delay (distance traveled and velocity). To increase the demands of the spatial task, the mice returned to the spatial task once again, 24hr later, and completed a probe trial (escape platform absent), and the time to reach the quadrant of the target platform location, as well as total time spent in the quadrant were recorded.
Results: Exposure to FLX during adolescence did not influence spatial memory acquisition on the training day. In addition, no differences between the groups were observed when spatial memory was examined 24 hr after training. On the other hand, mice exposed to FLX during adolescence swam longer distances to reach the location of the missing platform when tested 48 hr after training (probe trial).
Conclusions: Our results suggest that as the demands of the spatial memory task increase, spatial memory deficiencies become apparent in adult mice exposed to FLX during adolescence. Overall, these data underscore the need for further research for a clearer understanding of the long-term functional consequences of FLX exposure on the developing nervous system.
Keywords: Prozac Adolescence Spatial Memory SSRI
Disclosure: M. Stone, Nothing to Disclose; S. Nieto, Nothing to Disclose; T. Aiello, Nothing to Disclose; L. Riggs, Nothing to Disclose; S. Iñiguez, Nothing to Disclose.

M149. Subchronic Escitalopram Treatment Leads to SERT Internalization Rather than Down-regulation *in Vitro* and *ex Vivo*

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Background: Several observations using cell models or experimental animals suggest that subchronic treatment with selective serotonin transport inhibitors (SSRI) is associated with a down-regulation i.e. a reduced density of the serotonin transporter molecule (SERT) contributing to the enhanced serotoninergic neurotransmission as consequence of subchronic SSRI treatment. However, the mechanisms is not yet clear, especially the question if SERT molecules are down-regulated at the protein level or if only SERT availability for serotonin is reduced e.g. by enhanced internalization. This problem was addressed in the present study using escitalopram as the most selective SSRI and HEK cells stably expressing human SERT as *in vitro* model and mouse brain synaptosomes after subchronic treatment as *ex vivo* model.
Methods: For both conditions, SERT availability was investigated using ^{3}H-serotonin (^{3}H-5HT) uptake (HEK cells or mouse brain synaptosomes) and SERT density was determined by ^{3}H-citalopram binding to HEK cell or mouse brain membranes. *In vitro* treatment was carried out for 48 h using escitalopram 250 nM, R-citalopram 500 nM, and R,S-citalopram (500, 250 nM). For *ex vivo* experiments, NMRI mice were treated for 14 days with escitalopram (3,75 mg/kg), R-citalopram (7,5 mg/kg), or R-S-citalopram (7,5 mg/kg, 3,75 mg/kg) twice daily.
Results: Subchronic treatment of HEK cells with escitalopram reduced the maximal number of SERT sites by more than 50% as indicated by ^{3}H-5HT uptake experiments. R-citalopram was not active, while R,S-citalopram was similarly active as escitalopram. K_m of ^{3}H-5HT uptake was not different over all four groups. When ^{3}H-citalopram binding was determined using membranes of similarly treated HEK cells, no effect on B_{max} and K_D was seen for all three conditions relative to control cells. Similarly, treatment of mice with escitalopram reduced ^{3}H-5HT uptake site on synaptosomes by about 20% without altering K_m. Again R-citalopram was not, but R,S-citalopram was similarly active in reducing B_{max}. Km of ^{3}H-citalopram binding was not altered by either treatment.
Conclusions: The data obtained in both systems suggest that subchronic treatment with the SSRI escitalopram reduces the number of available SERT molecules at the cell surface (V_{max}) without having a significant effect on the total number of SERT molecules on the total protein level of the cells (B_{max}), most likely by enhanced internalization or intracellular sequestration. Escitalopram and R,S-citalopram were similarly active. The reduced activity of escitalopram in the presence of R-citalopram observed in many experiments *in vitro* and *in vivo* may not be associated with an amelioration of SERT disappearance at the cell surface after subchronic treatment (Supported by Lundbeck Copenhagen).
Keywords: escitalopram, SERT, down-regulation, chronic treatment
Disclosure: W. Mueller, **Part 1:** Seakers honorarium or scientific adviser for Lunfbeck, UCB, Schwabe, **Part 2:** Lundbeck, **Part 4:** Lundbeck, UCB, Schwabe, Cassellamed; J. Heiser, Nothing to Disclose; K. Leuner, **Part 1:** speakers honorarium UCB.

M150. An Examination of Involvement of the Dopamine Transporter (DAT), the Serotonin Transporter (SERT), and Monoamine Oxidase A (MAOA) in the Temperature and Locomotor Effects of 4-methylthiomethamphetamine (MTA)

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Background: 4-methylthiomethamphetamine (MTA) is a phenylisopropylamine derivative that can cause severe intoxication and death. MTA is a potent amphetamine derivative which causes serotonin release *in vitro* and *in vivo* mediated via the serotonin transporter (SERT). In addition, MTA also inhibits monoamine oxidase A (MAOA) and blocks the dopamine transporter (DAT). In order to further understand the mechanism(s) of action of MTA, we examined the effects of MTA on temperature and locomotion in wildtype C57BL/6J mice purchased from Jackson Laboratories, mice lacking the genes for *DAT*, *SERT or MAOA*, and wildtype (WT) littermate control animals.
Methods: Dose-response experiments examined the effects of 5, 10, 20, 40 or 80 mg/kg ip MTA versus saline on temperature and on locomotor activity in the open field in C57BL/6J mice. We next examined the temperature and locomotor effects of MTA (5 mg/kg ip) compared to vehicle (saline) in mice lacking DAT, SERT, or MAOA and their WT littermates.
Results: In C57BL/6J mice, MTA (5, 10 and 20 mg/kg) dose-dependently decreased temperature over time compared to baseline (time 0) and compared to saline-treated controls. Temperature change 30 min following MTA was significantly greater in mice administered 5, 10 or 20 mg/kg MTA compared to saline-treated controls (p's < 0.0001). Mice treated with 40 mg/kg MTA displayed an initial significant decrease followed by increasing temperature over time. All mice administered 80 mg/kg MTA died within $\sim$90 min following injection. MTA (5 mg/kg) significantly decreased temperature over time in DAT-WT, DAT-knockout (KO), MAOA-WT and MAOA-KO mice over time. Temperature change 30 min following MTA was similar in the two DAT genotypes (NS), while temperature change was significantly less in MAOA-KO mice compared to MAOA-WT mice ($p = 0.015$). Preliminary temperature data in SERT mice also suggest altered temperature effects of MTA in SERT-KO mice. In initial dose-response assessments in the open field, MTA (5, 10 and 20 mg/kg) significantly decreased horizontal locomotor activity and velocity in C57BL/6J mice in a dose-dependent manner compared to saline-treated mice. MTA (5, 10 and 20 mg/kg) significantly and dose-dependently decreased the number of visits to the center of the open field, suggesting increased anxiety-like effects. Further, the patterns of locomotion were altered in mice administered MTA, as meandering (the change in direction of movement of a subject relative to the

distance moved by that subject) was increased following MTA (5, 10 and 20 mg/kg) compared to saline-treated mice. In line with previous reports, compared with their respective WT controls, baseline locomotor activity was increased in DAT-KO mice and decreased in SERT-KO mice, and was slightly decreased in MAOA-KO mice. Preliminary open field data in mice lacking *DAT*, *SERT* or *MAOA* suggest interesting roles for these genes in MTA-induced changes in locomotor activity.
Conclusions: The present results suggest that the effects of MTA are mediated by its actions on multiple targets. Mechanisms of *in vivo* MTA actions are likely to involve multiple monoaminergic components. Current information about the neurochemical effects of MTA is relatively scarce when compared with other abused amphetamines including MDMA (methylenedioxymethamphetamine, 'ecstasy'). This relative promiscuity should be considered when assessing the global effects of this drug, in particular when used by humans.
Keywords: 4-methylthiomethamphetamine (MTA) Animal models Dopamine transporter (DAT) Monoamine oxidase A (MAOA) Serotonin transporter (SERT)
Disclosure: M. Fox, Nothing to Disclose; P. Moya, Nothing to Disclose; R. Sotomayor-Zárate, Nothing to Disclose; P. Iturriaga-Vásquez, Nothing to Disclose; F. Hall, Nothing to Disclose; K. Chen, Nothing to Disclose; J. Shih, Nothing to Disclose; G. Uhl, Nothing to Disclose; M. Reyes-Parada, Nothing to Disclose; D. Murphy, Nothing to Disclose.

M151. Decreasing Activity of Neuronal Nicotinic Receptors Can Improve Cognitive Function

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Background: Nicotine and other nicotinic agonists have been found to improve cognitive function, including attention and memory. However, since nicotinic receptors are easily desensitized, it is not clear to what degree this improvement is due to the agonist effect of these drugs and how much to the nicotinic receptor desensitization. Our lab and others have found that nicotinic receptor desensitization or outright blockade can significantly improve attention, learning and memory. In rats, the a4b2 nicotinic receptor desensitizing agent sazetidine-A significantly improved attentional function, reversing attentional impairment caused by blockade of muscarinic cholinergic receptors with scopolamine and blockade of NMDA glutamate receptors with dizocilpine (MK-801). In other studies, we found that low doses of the general nicotinic antagonist mecamylamine significantly improved learning and memory in the radial-arm maze. The current studies were conducted to determine the effects of specific a7 and a4b2 antagonists on attentional function.
Methods: Young adult Sprague-Dawley rats were trained on a visual signal detection operant task to assess attentional function. They were administered the nicotinic a7 antagonist nicotinic antagonist methyllycaconitine (MLA) or the a4b2 antagonist dihydro-b-erythroidine (DHbE) sc in doses of 1, 2, 4 or 8 mg/kg to determine the effects of decreasing nicotinic receptor activity on attention. In a follow-up study, the interaction of these antagonists (8 mg/kg) with the NMDA glutamate antagonist dizocilpine (0.05 mg/kg) and nicotine (0.025 and 0.05 mg/kg) were assessed. The vehicle saline was used as the control condition in both studies.
Results: The DHbE dose-effect function study showed a significant ($p < 0.005$) main effect of DHbE on total percent correct. All DHbE doses caused significantly ($p < 0.05$) higher accuracy than control vehicle. MLA dose-effect function study did not show a significant improvement in percent correct. In fact, there was a significant ($p < 0.05$) decrease in percent hit relative to control with the 1 mg/kg MLA dose. In the interaction study, dizocilpine significantly ($p < 0.005$) impaired choice accuracy. The interaction of DHbE x nicotine was significant ($p < 0.05$). The addition of 0.05 mg/kg nicotine to 8 mg/kg of DHbE significantly ($p < 0.005$) reduced accuracy relative to DHbE alone. The three-way interaction of DHbE x dizocilpine x nicotine was significant ($p < 0.05$). Tests of the simple main effects showed that dizocilpine caused a significant ($p < 0.005$) impairment in total percent correct relative to vehicle control. DHbE (8 mg/kg) co-administration with dizocilpine (0.0625 mg/kg) significantly ($p < 0.025$) reduced the dizocilpine-induced impairment in percent correct. Interestingly, the addition of nicotine (0.05 mg/kg) to DHbE significantly ($p < 0.0005$) reduced the ameliorative effect of DHbE in reversing the dizocilpine-induced impairment in percent correct. In the interaction study, the main effect of MLA was significant ($p < 0.005$) with MLA (8 mg/kg) improving accuracy and the main effect of dizocilpine significantly ($p < 0.0005$) impaired accuracy. The MLA x dizocilpine interaction was significant ($p < 0.005$). Tests of the simple main effects showed that dizocilpine caused a significant reduction in percent correct ($p < 0.0005$) and that MLA co-administration significantly ($p < 0.0005$) attenuated this impairment. Like DHbE, MLA attenuated the dizocilpine-induced impairment, but unlike DHbE the addiction of nicotine did not attenuate the beneficial effect of MLA of reversing the dizocilpine-induced accuracy impairment.
Conclusions: Acute administration of the a4b2 antagonist DHbE improved attentional function either alone or in reversing the attentional impairment caused by the NMDA glutamate antagonist dizocilpine. Acute administration of MLA also significantly attenuated the dizocilpine-induced attentional impairment. Decreasing nicotinic receptor activation improved attentional function and reversed dizocilpine-induced impairment. One key locus for this effect may be the mediodorsal thalamic nucleus, which has direct connections with the frontal cortex. We have found that acute or chronic local infusions of the a4b2 nicotinic antagonist DHbE into this area significantly improves working memory in the radial-arm maze. Development of drugs that provide a net decrease in nicotinic receptor activity either through antagonism or desensitization could be worth exploring for beneficial effects for treating cognitive impairments.
Keywords: nicotinic, attention, antagonists, MLA, DHbE
Disclosure: E. Levin, **Part 1:** AstraZeneca, Gilead, Astellas, Euthymics, Targacept, Memory Pharmaceuticals, **Part 4:** AstraZeneca, Gilead, Astellas, Euthymics, Targacept, Memory Pharmaceuticals; M. Cauley, Nothing to Disclose; A. Rezvani, **Part 1:** AstraZeneca, Gilead, Astellas, Euthymics, Targacept, Memory Pharmaceuticals, **Part 4:** AstraZeneca, Gilead, Astellas, Euthymics, Targacept, Memory Pharmaceuticals.

M152. CTP-354: A Novel Deuterated Subtype-selective GABA(A) Modulator for Treatment of Neuropathic Pain, Spasticity and Anxiety Disorders

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Background: $GABA_A$ receptors are a family of ligand-gated chloride channels that function as inhibitory neurotransmitter receptors in the CNS. The $GABA_A$ receptor is a pentameric protein with subtypes composed of α, β, and γ subunits. Typical benzodiazepines activate the receptor in a non-selective manner, binding to an allosteric site at the interface of a γ subunit and either an $\alpha1$, $\alpha2$, $\alpha3$ or $\alpha5$ subunit. Mutational studies in mice indicate that the sedative and ataxic effects of benzodiazepines are mediated by $\alpha1$ subtypes. Agonism at the $\alpha2$ and $\alpha3$ subtypes is believed to be associated with anxiolytic and spasmolytic activities, whereas $\alpha5$ subtype activity is believed to have cognitive effects.

Concert has prepared CTP-354, a non-benzodiazepine, as a promising subtype-selective $GABA_A$ modulator for neuropathic pain, spasticity and anxiety disorders. CTP-354 is an analog of L-838417, which was evaluated preclinically as part of Merck's research effort towards subtype-selective $GABA_A$ anxiolytics with reduced sedation and ataxia. L-838417 was reported to possess a particularly attractive subtype-selective $GABA_A$ pharmacologic profile, with agonism at $\alpha 2$ and $\alpha 3$ and antagonism at $\alpha 1$. However, despite its desirable $GABA_A$ subtype selectivity, publications from Merck indicated that L-838417 possessed a poor preclinical pharmacokinetic profile and therefore was not advanced into clinical development. Recently, L-838417 was reported to be efficacious in preclinical models of inflammatory and neuropathic pain, and to exhibit strong muscle relaxant effects, further expanding the potential therapeutic utility of the compound beyond anxiolysis. CTP-354 was designed to overcome the poor PK of L-838417 by incorporating deuterium atoms in place of hydrogen at key positions. Deuterium effects on metabolism are unpredictable, even when deuterium is inserted at a known site of metabolic oxidation. In select cases, however, deuterium substitution can significantly improve a drug's metabolic properties while preserving its pharmacological activity. Concert has designed and synthesized a number of novel deuterated L-838417 analogs with enhanced metabolic stability. We have compared our precision-deuterated analogs to L-838417 with respect to *in vitro* metabolic stability and *in vivo* pharmacokinetics, and CTP-354 has been selected as our lead compound. We have progressed CTP-354 into animal models of sedation/ataxia and neuropathic pain.

Methods: CTP-354 and L-838417 were compared in *in vitro* and *in vivo* DMPK assays and pharmacology studies. *In vitro* assessment of metabolic stability was conducted in Sprague-Dawley (SD) rat liver microsomes (RLM, 2 mg/mL) and human liver microsomes (HLM, 2 mg/mL) over 30 min at a compound concentration of 0.25 μM. The PK parameters of CTP-354 and L-838417 were compared in discrete-dose studies in male SD rats ($n = 8$, 1 mg/kg) and in male beagle dogs ($n = 4$, cross-over study, 15 mg/kg) dosed orally. CTP-354 and L-838417 were evaluated at 10 μM in the *in vitro* Ricerca LeadProfilingScreen®, a standard selectivity screen of 68 primary targets including GPCRs, ion channels, CNS transporters, and enzymes. CTP-354 was assessed in a rat rotarod model at oral doses up to and including 100 mg/kg to ascertain sedation/ataxia liability via latency to fall from rod. The Chung model, a sciatic nerve ligation model of neuropathic pain, was performed in the SD rat to compare CTP-354 and L-838417 at oral doses up to 10 mg/kg. Von Frey fibers were used to assess paw withdrawal response for the affected paw and contralateral paw. A follow-up study compared doses of CTP-354 up to 100 mg/kg versus gabapentin at 100 mg/kg as a standard-of-care positive control.

Results: *In vitro* metabolic stability assessment in RLM and in HLM showed CTP-354 was highly stabilized versus L-838417. *In vivo* oral PK studies comparing CTP-354 to L-838417 in the rat and dog demonstrated a 3- to 4-fold increase in exposure for CTP-354. In the Ricerca selectivity screen CTP-354 and L-838417 showed binding to the benzodiazepine site of the $GABA_A$ receptor, as expected, with no significant off-target activities. CTP-354 was well tolerated in the rat rotarod model at oral doses up to and including 100 mg/kg. In the rat Chung model of neuropathic pain CTP-354 was efficacious and demonstrated a significantly prolonged pharmacodynamic effect versus L-838417 at the 10 mg/kg dose. In the second Chung model study, with doses up to 100 mg/kg, CTP-354 showed a dose response and demonstrated equivalent efficacy to gabapentin with a superior duration of effect.

Conclusions: CTP-354 has shown markedly improved metabolic stability relative to L-838417 in both *in vitro* and *in vivo* DMPK assessments. CTP-354 has been evaluated in rodent *in vivo* efficacy models assessing sedation/ataxia and amelioration of neuropathic pain, demonstrating a lack of sedative/ataxic effects at doses which afford excellent pain protection. CTP-354 has demonstrated equivalent efficacy to gabapentin in the Chung model with an enhanced duration of effect. Based on this favorable profile, CTP-354 has been selected as a development candidate and is undergoing IND-enabling non-clinical toxicology and safety pharmacology studies.

Keywords: GABAA, pain, spasticity, anxiety, GABA(A)

Disclosure: J. Liu, **Part 1:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 2:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 3:** Full-time employee of Concert Pharmaceuticals, Inc.; S. Harbeson, **Part 1:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 2:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 3:** Full-time employee of Concert Pharmaceuticals, Inc.; V. Uttamsingh, **Part 1:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 2:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 3:** Full-time employee of Concert Pharmaceuticals, Inc.; A. Morales, **Part 1:** Formerly a full-time employee of Concert Pharmaceuticals, Inc. Currently a full-time employee of Novartis, **Part 2:** Full-time employee of Novartis, **Part 3:** Full-time employee of Novartis; S. Nguyen, **Part 1:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 2:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 3:** Full-time employee of Concert Pharmaceuticals, Inc.; G. Bridson, **Part 1:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 2:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 3:** Full-time employee of Concert Pharmaceuticals, Inc; C. Cheng, **Part 1:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 2:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 3:** Full-time employee of Concert Pharmaceuticals, Inc.; A. Aslanian, **Part 1:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 2:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 3:** Full-time employee of Concert Pharmaceuticals, Inc.; L. Wu, **Part 1:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 2:** Full-time employee of Concert Pharmaceuticals, Inc., **Part 3:** Full-time employee of Concert Pharmaceuticals, Inc.

M153. Activation of Metabotropic Glutamate Receptor 7 (mGluR7) by AMN082 Attenuates the Rewarding Effects of Cocaine and Nicotine in Rats

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Background: The metabotropic glutamate receptor 7 (mGluR7) has received much attention as a potential target for the treatment of epilepsy, depression, anxiety and, most recently, drug dependence. In the present study, we investigated the role of mGluR7 in cocaine and nicotine reward based upon our previous findings that activation of mGluR7 by the selective agonist AMN082 modulated glutamatergic neurotransmission in the nucleus accumbens (NAc), a critical reward-related brain region.

Methods: The intravenous self-administration rat procedure was used to study the positive reinforcing and motivational effects of cocaine and nicotine.

Results: The results indicated that systemic administration of AMN082 (3, 10, 20 mg/kg, intraperitoneal [i.p.]) decreased cocaine and nicotine self-administration under fixed ratio (FR) and progressive ratio (PR) schedules of reinforcement, indicating that AMN082 inhibited both the primary reinforcing and incentive motivational effects of cocaine and nicotine. Most interestingly, 3 mg/kg AMN082, a dose that did not induce a statistically significant reduction in cocaine self-administration under an FR2 schedule of reinforcement, significantly inhibited nicotine self-administration under an FR5 schedule. A plausible explanation for the increased sensitivity of nicotine self-administration, relative to cocaine self-administration, to the effects of AMN082 is that because both mGluR7 and nicotinic acetylcholine receptors

(nAChRs) are located on glutamatergic neurons in the ventral tegmental area (VTA), the interaction between mGluR7 and nAChRs activation may be more direct and effective than interactions between mGluR7 and the primary sites of action of cocaine (i.e., monoamine transporters). Importantly, AMN082 did not affect sucrose consumption at doses that exhibited effectiveness in models of drug dependence in rats, suggesting that AMN082 has no effects on the rewarding properties of non-drug reinforcers.
Conclusions: These findings suggest that mGluR7 may be a promising target for the treatment of cocaine and nicotine dependence with few undesirable side-effects.
Keywords: mGluR7, cocaine, nicotine, self-administration
Disclosure: X. Li, Nothing to Disclose; A. Stoker, Nothing to Disclose; Z. Xi, Nothing to Disclose; E. Gardner, Nothing to Disclose; A. Markou, Nothing to Disclose.

M154. Lithium Potentiates Long-Term Synaptic and Antidepressant-Like Effects of Ketamine

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Background: Previous studies in normal or stressed rodents show that the rapid antidepressant action of a single subanesthetic dose of the NMDA antagonist ketamine is associated with an increase in synapse formation/maturation (synaptogenesis) in medial prefrontal cortex (mPFC); these synaptic changes are mediated in part by activity-dependent stimulation of the BDNF/mTORC1 pathway (see Li et al., 2010/2011, Liu et al., 2012). Associated with ketamine's antidepressant effect is an increase in large amplitude EPSCs and large, stable (mushroom) spines. As GSK3 inhibitors are known to promote synaptic stabilization by reducing long-term synaptic depression ("synaptosis"; Bradley et al, 2012), we investigated whether co-administration of a low dose of lithium, which has GSK3 inhibitory activity (see Gould and Manji, 2005), or a selective GSK3 inhibitor would potentiate the long-term synaptogenic and antidepressant effects of ketamine.
Methods: Adult rats were given a single injection of ketamine, either a standard (10 mg/kg, i.p.) dose or low dose (1 mg/kg, i.p.) alone or in combination with lithium chloride (10 mg/kg, i.p.; 30 min following ketamine). After twenty-four hours or 1 week, whole-cell patch clamp recordings were made from layer V pyramidal cells in mPFC brain slices. Serotonin (5-HT) and hypocretin /orexin-induced EPSCs were recorded; the recorded cells were filled with Neurobiotin and later imaged by 2-photon a laser scanner. Another cohort of animals was used for analysis of mTOR signaling by western blot and behavior in the forced swim test (FST).
Results: In contrast to a 10 mg/kg dose of ketamine (Li et al., 2010), a low dose of ketamine (1 mg/kg) was ineffective in inducing a synaptogenic effect. However, in combination with a single dose of lithium, the synaptogenic and EPSP-enhancing effect of low-dose of ketamine was potentiated to a level comparable to high-dose ketamine both at 24 hrs and 1-week post injection. A selective GSK3 inhibitor had similar potentiating effects on low-dose ketamine. In parallel studies, lithium was demonstrated to enhance the effects of low-dose ketamine on mTOR signaling and immobility in the FST.
Conclusions: Potentiation of the long-term synaptogenic and antidepressant effects of an otherwise sub-threshold dose of ketamine by co-administration of lithium or a GSK3 inhibitor is consistent with data showing that antidepressant-like effects of ketamine are dependent in part on increased inhibitory phosphorylation of GSK3 (Beurel et al., 2011). This is the first analysis comparing long-term synaptogenic and antidepressant effects of low-dose ketamine in combination with lithium. The potentiation of sub-threshold ketamine by lithium may have clinical usefulness in avoiding the dissociative or toxic effects of higher doses of ketamine as currently used in single or repeated treatments. Li et al., Science, 2010; Biol Psychiatry 2011 Liu et al., Biol. Psychiatry, 2012. Bradley et al., Mol. Neurosci. 2012. Gould and Manji, Neuropsychopharmacology 2005. Beurel et al., Mol. Psychiatry, 2011.
Keywords: synaptogenic ketamine lithium antidepressant GSK3
Disclosure: R. Liu, Nothing to Disclose; M. Fuchikami, Nothing to Disclose; J. Dwyer, Nothing to Disclose; R. Duman, Nothing to Disclose; G. Aghajanian, Nothing to Disclose.

M155. Identification of a Novel Dopaminergic Agonist that Selectively Activates the D2 Dopamine Receptor

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Background: The D_2 dopamine receptor (DAR) is central in the etiology and/or therapy of many neuropsychiatric disorders. Specifically, D_2 DAR antagonism is the hallmark of all FDA-approved antipsychotics and stimulation of the D_2 DAR is critical for effective antiparkinsonian therapy. Unfortunately, truly specific drugs for this receptor have been difficult to obtain, primarily due to high conservation of the orthosteric binding site within DAR subtypes and between other G protein-coupled receptors. In order to develop novel molecular scaffolds for the D_2 receptor, we used high throughput screening to interrogate a small molecule library to identify hit ligands with distinct functional characteristics, mechanisms of action, and selectivity among DAR subtypes. This process led to the identification of a ligand (compound 3508) that selectively activates the D_2 DAR in comparison with other DAR subtypes.
Methods: We developed a high throughput-screening (HTS) platform to interrogate large chemical compound libraries and have screened a 370,000+ small molecule library to identify agonists, positive allosteric modulators, or antagonists. The primary HTS assay utilizes a cell line expressing the D_2 DAR coupled to a chimeric Gqi5 protein, thereby linking receptor activation to robust Ca^{2+} mobilization that is measured using a fluorescent readout. We have also developed HTS-formatted secondary assays to measure orthogonal D_2 DAR activities (cAMP modulation, β-arrestin interactions, GIRK channel activation) as well as counter-screening assays to determine selectivity between other DAR subtypes (D_1, D_3, D_4 and D_5). Effects of hit ligands on the orthosteric binding site of the D_2 DAR was assessed using standard radioligand binding competition assays.
Results: The primary HTS screen resulted in the identification of ~2,288 compounds with agonist activity and ~2,294 compounds with antagonist activity. These compounds were cherry-picked to maximize the chances of identifying chemical series with unique activities. Hits were subjected to orthogonal and counter-screening functional assays. While the primary goal for this screen was to identify allosteric compounds, a by-product of this screen and subsequent triaging was the identification of compounds that, while potentially orthosteric, exhibit high selectivity for the D_2 DAR and/or are functionally selective with respect to D_2 DAR signaling pathways. One such chemotype discovered this way (compound 3508) selectively activates the D_2 DAR in comparison with other DAR subtypes. We found that compound 3508 exhibits full agonist activity with EC_{50} values ranging from 100 nM – 1 μM using three different functional assays for the D_2 DAR; Ca^{2+} mobilization, inhibition of cAMP accumulation, and β-arrestin recruitment. Using β-arrestin recruitment assays to compare with other DARs, we found that compound 3508 has no activity

at D1-like DARs (D_1 and D_5) or on D_4 DARs. However, compound 3508 displays either weak partial agonist (<20% of the DA response) or full antagonist activity at D_3 DARs depending on the assay. We also studied compound 3508 using a G protein-based Go BRET activation assay. Compound 3508 was a full agonist at the D_2 DAR but displayed only weak partial (<20%) agonist activity at the D_3 DAR. Interestingly compound 3508 is a full antagonist with no agonist activity on D_2 DAR-linked GIRK channel activation indicating that it is a functionally-selective, or biased agonist at the D_2 DAR. Radioligand binding assays revealed that compound 3508 exhibits Ki values of ~1 μM and ~100 nM for the D_2 and D_3 DARs, respectively. Molecular modeling studies suggest subtle differences in how compound 3508 docks to the D_2 and D_3 DARs that might explain the differences in its functional properties.
Conclusions: In summary, compound 3508 is a full and selective agonist at G-protein-linked and arrestin mediated D_2 DAR assays; however, it shows antagonist activity in D_2 GIRK channel assays suggesting that it is a biased D_2 DAR agonist. Furthermore, it generally functions as a potent D_3 DAR antagonist in functional assays. This is the first known compound that functionally discriminates between the D_2 and D_3 DARs in that it selectively and fully activates the D_2 DAR while antagonizing D_3 DAR activation.
Keywords: dopamine D2 D3 receptor agonist
Disclosure: D. Sibley, Nothing to Disclose; R. Free, Nothing to Disclose; J. Conroy, Nothing to Disclose; R. Roof, Nothing to Disclose; T. Doyle, Nothing to Disclose; N. Southall, Nothing to Disclose; M. Ferrer, Nothing to Disclose; P. Donthamsetti, Nothing to Disclose; M. Michino, Nothing to Disclose; Y. Han, Nothing to Disclose; L. Shi, Nothing to Disclose; J. Javitch, Nothing to Disclose.

M156. A Novel Nociceptin-1 Receptor Antagonist Produces Antidepressant-, Anxiolytic-, and Anti-Ethanol-Associated Effects in Rodent Models

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Background: Nociceptin/Orphanin FQ (Noc/OFQ) is a 17 amino acid peptide identified in 1995 whose receptor is designated ORL1 or NOP. Since then, some agonists and antagonists for the receptor have been designed and employed to help elucidate its biological functions. We now report on a highly potent, selective, and orally-bio-available antagonist with documented engagement with NOP receptors *in vivo*.
Methods: Compound 1 was evaluated in a host of *in vitro*, *ex vivo*, and *in vivo* assays to characterize its value as a NOP receptor antagonist research tool and to define its biological signature relating to potential therapeutic options. These include functional activity at NOP receptors, occupancy of NOP receptors *in vivo*, andeffects on behavior and neurochemistry in rodent models that detect antidepressants, anxiolytics, and drugs that suppress ethanol intake.
Results: Compound 1 has a K_b of 0.17 nM as an antagonist of Noc/OFQ-stimulated G protein activation. The molecule is CNS penetrant and occupies central Noc/OFQ receptors with high potency and duration. *In vivo* activity was demonstrated by its potent inhibition of CNS-mediated hypothermic effects of a Noc/OFQ agonist. The NOP receptor antagonist, Compound 1, displayed antidepressant-like neurochemical and behavioral effects that were consistent with a burgeoning body of data. Serotonin outflow in rat prefrontal cortex was enhanced by Compound 1 and the compound correspondingly decreased immobility in the forced swim test in mice and rats. Compound 1 also augmented the effects of fluoxetine without changing brain or plasma levels of either drug alone. Anti-immobility effects of Compound 1 were absent in mice without NOP receptors while the anti-immobility effects of imipramine were spared. In contrast to literature predictions, however, Compound 1 also produced anxiolytic-like effects in some rodent models. Moreover, although the literature had suggested that NOP receptor agonists would attenuate alcohol self-adminstration by rodents, the NOP receptor antagonist Compound 1 attenuated both neurochemical and behavioral effects of ethanol in rodent models.
Conclusions: These data suggest that Compound 1 can function as an orally-active tool for assessments of the functional significance of NOP receptors. The current sets of findings in rodents are consistent with anti-depressant and anti-anxiety efficacy along with the ability to attenuate ethanol-driven effects in rodents.
Keywords: NOP receptors, nociceptin, antidepressants, ethanol, rodents
Disclosure: J. Witkin, Nothing to Disclose; M. Statnick, Nothing to Disclose; D. Mckinzie, Nothing to Disclose; L. Rorick-Kehn, Nothing to Disclose; V. Barth, Nothing to Disclose; J. Pintar, Nothing to Disclose; K. Perry, Nothing to Disclose; M. Toledo, Nothing to Disclose; N. Diaz, Nothing to Disclose; C. Lafuente, Nothing to Disclose; A. Jimenez, Nothing to Disclose; M. Martinez-Grau, Nothing to Disclose.

M157. Preclinical Studies of the Multimodal Antidepressant Vortioxetine Support a Potential for Improvement of Cognitive Functions

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Background: The multimodal investigational antidepressant vortioxetine is a 5-HT_3 and 5-HT_7 receptor antagonist, 5-HT_{1B} receptor partial agonist, 5-HT_{1A} receptor agonist and inhibitor of the 5-HT transporter (SERT) *in vitro*. Combination of *in vitro* affinities at human and rat targets (receptors and SERT), dose-occupancy relation in rat brain (*ex vivo* autoradiography) and dose SERT occupancy relation from human PET studies support dose dependent occupancy of all targets at clinical doses of vortioxetine. The literature indicates that brain localization and physiological functions of vortioxetine's receptor mechanisms have the potential to enhance cognitive function. In rat microdialysis studies we have shown that vortioxetine dose-dependently increases extracellular 5-HT, NE, DA, acetylcholine and histamine in cortex[1,2,3], which may lead to enhanced cognitive functions. We have also shown in behavioural studies in rats that vortioxetine reverses time-induced deficits in contextual and episodic memory[3]. Finally, a clinical study in elderly depressed patients showed superiority of vortioxetine compared to placebo in cognition tests of speed of processing, verbal learning and memory[4]. Here we further explore vortioxetine's cognitive enhancing effects and its underlying mechanism of action in quantitative EEG analyses in rats and behavioural studies of memory deficits in 5-HT depleted rats.
Methods: Quantitative EEG: Male Sprague Dawley rats implanted with EEG (frontal and parietal cortex) and EMG (dorsal neck muscles) electrodes connected to a subcutaneously implanted multi-channel telemetric device were used. EEGs were recorded 90 min pre- to 4 h post-dosing (~3 h into light cycle) and vigilance states were determined as active and quiet wake and non-REM and paradoxical sleep using conventional criteria. Here we report the results of a spectral frequency (1-50 Hz) analysis of drug effects based on the 30-60 min pre- and 60-90 min post-dosing period for each rat. **Memory deficits in 5-HT depleted rats:** Female Long Evans rats were dosed daily with the tryptophan hydroxylase inhibitor, 4-chloro-DL-phenylalanine methyl ester HCl (PCPA, 85.5 mg/kg, sc) for 4 days and tested in novel object recognition

and spontaneous alternation tests of episodic and spatial memory, respectively, and the forced swim test model of depression-like behavior. Total 5-HT after PCPA was determined in brain hippocampal tissue and extracellular 5-HT was determined by microdialysis in the ventral hippocampus. 5-HT concentrations were determined by means of HPLC with electrochemical detection. Doses of vortioxetine, escitalopram, duloxetine, flesinoxan (5-HT_{1A} receptor agonist), ondansetron (5-HT_3 receptor antagonist) and SB 269970 (5-HT_7 receptor antagonist) were chosen based on target occupancies determined by *ex vivo* autoradiography.
Results: Vortioxetine at clinically equivalent doses showed dose-dependent increases in cortical theta, alpha and gamma power. Escitalopram had no effect while duloxetine (80-90% SERT occupancy) decreased alpha power significantly. Flesinoxan, ondansetron and SB 269970 increased power bands. Thus it is plausible that these receptor mechanisms contribute to vortioxetine's net effect. In PCPA treated rats, vortioxetine reversed episodic and spatial memory deficits at all clinically equivalent doses; while escitalopram and duloxetine (80-90% SERT occupancy) were inactive. Flesinoxan (30% 5-HT_{1A} receptor occupancy) reversed episodic and spatial memory deficits and ondansetron (25% 5-HT_3 receptor occupancy) reversed episodic memory. The effects on PCPA-induced memory deficits were sustained after vortioxetine dosing for 14 days in food. PCPA-treatment resulted in approximately 90 and 70% reduction in levels of hippocampal tissue and extracellular 5-HT, respectively. The 5-HT releaser, fenfluramine, increased extracellular 5-HT approximately 1000% whereas vortioxetine had no 5-HT releasing effect. Thus it is plausible that 5-HT_3 and 5-HT_{1A} receptor activity and not SERT inhibition contribute to vortioxetine's net effect in this model.
Conclusions: The dose-dependent increase of qEEG power indicates a role for vortioxetine in modulating cortical networks recruited during cognitive behaviors. Cortical theta activity is linked to the septohippocampal cholingeric system and involved in sensorimotor integration. Cortical alpha waves are mainly generated by cortical (layer IV-V) networks and are involved in recruitment/synchronization of neurons to generate a coordinated cortical output. Gamma oscillations are controlled by networks of inhibitory GABAergic interneurons acting at soma and dendrites of pyramidal neurons, which leads to a uniformed firing pattern of cortical networks. Vortioxetine's increase of qEEG power and reversal of memory deficits induced by low brain 5-HT levels indicate a different mechanism of action for vortioxetine compared to monoamine transporter inhibitors such as escitalopram and duloxetine and suggest significant contributions of its direct receptor activity to its net pharmacological effects. In conclusion, preclinical studies of vortioxetine support a potential role for the compound in enhancing cognitive functions in humans
References 1: Mørk et al. J Pharm Exp Ther, 2012, 304, 666-75. **2.** Pehrson et al, http://dx.doi.org/10.1016/j.euroneuro.2012.04.006. **3.** Mørk et al, Biol Psychiatr, 2012, 71.8, 299S-300S. **4.** Katona et al, Int Clin Psychopharmacol, 2012, 27, 215-23x
Keywords: Lu AA21004, multimodal, antidepressant, qEEG, memory, rat
Disclosure: S. Leiser, **Part 1:** Employee of Lundbeck Research USA; A. Pehrson, **Part 1:** Employee of Lundbeck Research USA; P. Robichaud, **Part 1:** Employee of Lundbeck Research USA; K. Nielsen, **Part 1:** Intern at Lundbeck Research USA; J. Jensen, **Part 1:** Intern at Lundbeck Research USA; G. Smagin, **Part 1:** Employee of Lundbeck Research USA; D. Song, **Part 1:** Employee of Lundbeck Research USA; D. Budac, **Part 1:** Employee of Lundbeck Research USA; A. Frazer, **Part 1:** H Lundbeck A/S & Takeda Pharmaceutical Company Ltd, Advisory Board, Eli Lily, Advisory Board; C. Sanchez, **Part 1:** Employee of Lundbeck Research USA.

M158. The Insoitol Depletion Hypothesis of Lithium's Mechanism of Action is Not Dead; Lithium Affects Mouse Brain Inositol Turnover with Behavioral Consequences in Bipolar-related Paradigms

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Background: The therapeutic mechanism of action of lithium is not yet resolved despite numerous findings of biochemical and cellular changes induced by the drug. Among the latter lithium's inhibition of human inositol monophosphatase (IMPase)1 led to the inositol depletion hypothesis suggesting that lithium's mood-stabilizing action is mediated through an effect on inositol metabolism. While the role of inositol metabolism in lithium's action has gained substantial support it also presents serious concern: (*i*) there are reports of studies which failed to demonstrate reduced brain inositol levels following chronic or acute lithium treatment of patients or animals, (*ii*) the effect of lithium on IP_3 levels is species-dependent, resulting in either reduced or increased levels, (*iii*) IMPA1 (the gene that encodes for IMPase1) KO mice did not show reduced frontal cortex or hippocampal inositol levels although their IMPase activity was reduced by ~50%, (iv) sodium *myo*-inositol transporter (SMIT)1 homozygote KO mice in which brain inositol levels are ~60% reduced do not exhibit the expected reduction in brain phosphatidilinositol levels. Mammalian inositol's metabolism is well characterized but the regulation of its turnover has not been elaborated. In agreement with the inositol depletion hypothesis IMPA1 homozygote knockout mice exhibit lithium-like behavior in the forced-swim test and the pilocarpine-induced seizures paradigm. Since the inositol signaling pathway is cyclic requiring continuous incorporation of inositol into the membranal phosphatidylinositol pool, understanding brain inositol's turnover and how lithium affects it is crucial for better understanding the consequences of inositol depletion. We hypothesized that lithium dampens brain inositol turnover and that bi-allelic IMPA1 knockout results in a similar effect.
Methods: We used intracerebroventricular (icv) administration of ^{3}H-inositol to determine inositol turnover into brain phosphoinositols and phosphoinositides. Phosphoinositols were separated by anion exchange chromatography and phosphoinositides - by chloroform:methanol:HCl (100:200:1) extraction. IP_3 was administered icv in liposomes.
Results: Acute and chronic lithium treatment as well as ablation of IMPA1 resulted in a several fold increase in radiolabeled phosphoinositols in the hippocampus and in the frontal cortex while there was no change in radiolabeled phosphoinositides in both brain areas. Assuming that increased total phosphoinositols reflects increased IP_3 levels we studied if icv administration of IP_3 induces lithium-like effects. Administration of IP_3 in liposomes icv resulted in a lithium-like decreased immobility time in the forced-swim test and attenuated hyperlocomotion response to amphetamine.
Conclusions: The biochemical and behavioral results support the inositol depletion hypothesis of lithium's mechanism of action but suggest that inositol's metabolic flux (turnover) rather than inositol levels is the critical factor which mediates mood stabilization as modeled in behavioral paradigms of depression and mania. It is of interest to further investigate the molecular mechanism which mediates the behavioral consequences of IP_3 accumulation due to the decrease in inositol turnover. It is worth noting that lithium has been shown to induce autophagy in an mTOR-independent manner that is controlled by IP_3 receptor which might be activated by the accumulating IP_3.
Keywords: lithium, Inositol turnover, IMPA1 knockout mice, behavior
Disclosure: G. Agam, Nothing to Disclose; Y. Sade, Nothing to Disclose.

M159. Altered Tonic and Phasic Glutamate in Prelimbic and Infralimbic Prefrontal Cortices of Anesthetized and Awake SHR Rats

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Background: Recent clinical research has implicated glutamate in the etiology of attention-deficit/hyperactivity disorder (ADHD) and drug abuse. Using proton magnetic resonance spectroscopy, it was discovered that children and adults with ADHD exhibited increased levels of a marker for glutamine/glutamate in the prefrontal cortex (PFC). The stimulant medication methylphenidate, a dopamine transporter reuptake inhibitor, was found to lower these levels (Moore et al, 2006, Hammerness, et al., 2012). Furthermore, recent data from our laboratory suggests that high doses of methylphenidate (10 mg/kg) decrease glutamate levels in the prelimbic and infralimbic cortices within the PFC. Previous evaluations of a rodent model of ADHD, the spontaneously hypertensive rat (SHR/NCrl), found that the SHR has increased AMPA receptor activity and elevated calcium levels in the PFC, suggesting that altered glutamatergic neurotransmission may exist in the PFC of the SHR (Lehohla et al., 2004). We hypothesized that increased glutamate levels exist in prefrontal cortex subregions of the SHR compared to the SHR progenitor control, the Wistar Kyoto (WKY/NHsd) and that methylphenidate would reduce glutamate levels.
Methods: All studies were performed using constant potential amperometry with the FAST16mkIII system (Quanteon LLC). For anesthetized depth profile experiments, glutamate oxidase-coated S2 Microelectrode Arrays (MEAs) (4 Pt sites measuring 15 x 333 mm arranged vertically in dual pairs) were used as previously described (Hinzman, et al., 2012). A size exclusion layer of 1,3–phenylenediamine (mPD) was electroplated onto the Pt sites to block large molecule interferents, such as ascorbic acid and dopamine, from reaching the recording sites. Briefly, the MEA was lowered through the striatum and PFC in 500 mm increments and tonic glutamate and KCl-evoked glutamate release were examined. For freely-moving experiments, the novel DSPR8 (Double-Sided Paired Row 8 channel) MEA was used. These MEAs consist of four pairs of Pt recording sites with four sites, arranged vertically and at the same level, on each side of the ceramic substrate. The Pt recording sites are 50 x 100 μm with 100 μm spacing between the ceramic tip and the lower recording sites. One side of the MEA was coated with the enzyme glutamate oxidase which allows for a self-referenced glutamate signal with the non-coated side. Before each DSPR8 implantation surgery, an *in vitro* calibration was performed to ensure that the MEA was sensitive and selective for glutamate. Eight week old male SHR and WKYs were anesthetized with isoflurane (2-3%) and the DSPR8 MEA was inserted into the brain (AP: +3.2 mm; ML: −0.8 mm; from bregma). A reference electrode was inserted into the contralateral hemisphere. The MEA and reference electrode were secured with dental cement. Recording sites were located in the dorsal peduncular cortex, the infralimbic cortex, the prelimbic cortex and the cingulate cortex. Glutamate recordings were obtained at 10 Hz for 10 consecutive days with a daily schedule of: 3 hours of pre-treatment recording beginning at 0900 (during the light cycle), a 2 mg/kg injection of MPH administered s.c., followed by 3 hours of post-treatment recording. Rats were free to move around as the recording apparatus was suspended by a tethered cord. Behavior was recorded using Digiscan Animal Monitoring activity boxes (Omnitech Inc.). Resting glutamate levels pre- and post-treatment were calculated, as well as glutamate transients during each recording period. All data were analyzed using t-tests followed by Bonferroni post-hoc comparisons.
Results: Depth profile experiments in anesthetized animals were first used to isolate glutamatergic differences between the SHR and WKY before chronic implantation of the DSPR8 MEAs. We discovered that the SHR model of ADHD displayed significantly increased tonic glutamate in the prelimbic cortex in SHR (3.6 ± 0.8 μM in SHR, 1.4 ± 0.2 μM in WKY, $p < 0.05$), and increased KCl-evoked glutamate release in the prelimbic and infralimbic cortices compared to control (prelimbic: 20.3 ± 2.3 μM in SHR, 10.7 ± 1.2 μM in WKY, $p < 0.001$; infralimbic: (17.1 ± 2.2 μM in SHR, 7.6 ± 1.8 μM in WKY, $p < 0.01$). We also found increased tonic and KCl-evoked glutamate levels in the striatum and nucleus accumbens core (data not shown), providing further evidence of glutamatergic dysfunction in the SHR. Next, we recorded second-by-second glutamatergic neurotransmission in multiple PFC subregions simultaneously while recording locomotion with the open-field activity boxes. Freely-moving measures demonstrated differences in glutamate levels and locomotion pre- and post-methylphenidate treatment.
Conclusions: We have demonstrated elevated tonic and phasic glutamate in the prefrontal cortices of the SHR, which correlates with similar findings in the DRD4 knockout mouse and MRI spectroscopy studies in humans with ADHD. These findings suggest that glutamate regulation may be a potential target for pharmacotherapy in ADHD and perhaps stimulant drug abuse. Simultaneous measurements of behavior and second-by-second glutamate present novel possibilities for future experiments.
Keywords: Glutamate methylphenidate prefrontal ADHD stimulant
Disclosure: P. Glaser, **Part 1:** I have received research funds to perform clinical trials with Eli Lilly in the fields of Anxiety, Bipolar, and Schizophrenia research, **Part 4:** I have received research funds to perform clinical trials with Eli Lilly in the fields of Anxiety, Bipolar, and Schizophrenia research; E. Miller, Nothing to Disclose; F. Pomerleau, Nothing to Disclose; G. Gerhardt, **Part 1:** I own the company, Quanteon LLC, that produces the electrodes and recording equipment used in this study.

M160. Evidence for Involvement of Nitric Oxide and GABA-B Receptors in MK-801-Stimulated Glutamate Efflux in the Rat Prefrontal Cortex

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Background: Extracellular glutamate in hte prefrontal cortex (PFC) is dramatically elevated following treatment with NMDA antagonists, e.g., MK-801, phencyclidine. Although the mechanisms underlying this glutamate response are not entirely elucidated, evidence suggests events downstream from NMDA receptor blockade are linked to outcome measures of direct relevance to schizophrenia. Importantly, antipsychotic agensts prevent NMDA antagonist-induced elevations in extracellular glutamate in the PFC and impairments in prepulse inhibition and learning and memory. Recent studies provide evidence supportive of a role of nitric oxide (NO), as well as $GABA_B$ receptors, in the dirsuption of prepulse inhibition evoked by NMDA antagonists. In the present study, we have investigated the involvement of NO and $GABA_B$ receptors in the MK-801-induced increse in glutamte efflux in the PFC.
Methods: *In vivo* microdialysis was employed to quantify extracellular concentrations of glutamate in the PFC. Dialysis probes were inserted through guide cannulae that had been implanted 72 hr earlier. The probes were perfused overnight with Dulbecco's phosphate buffered saline prior to the start of the experiment. Glutamate in OPA derivatized dialysis samples was quantified by HPLC with electrochemical detection.
Results: The systemic administration of MK-801 (0.3 mg/kg, sc) significantly increased glutamate efflux in the PFC. This response was markedly attenuated in rats treated with the NO synthase inhibitor L-NAME. Reverse dialysis of the NO donor SNAP directly

into the PFC dose dependently increased glutamate efflux. Finally, the stimulatory effect of MK-801 on glutamate efflux was significantly suppressed by the systemic administration of the $GABA_B$ agonist baclofen.
Conclusions: The results of the present study support the view that increased NO formation is necessary for NMDA antagonist induced elevation in extracellular glutamate in the PFC. Moreover, it appears that increased NO availability *per se* is sufficient to increase glutamate efflux. THe data further support the conclusion that $GABA_B$ receptors can modulate cortical glutamate efflux. It is tempting to speculate that the ability of NOS inhibitors and $GABA_B$ agonists to suppress the behavioral effects of NMDA antagonists is releated to their effects to suppress NMDA antagonist evoked glutamate release.
Keywords: glutamate, NMDA antagonist, nitric oxide, GABA-B receptoprs
Disclosure: G. Gudelsky, Nothing to Disclose; N. Roenker, Nothing to Disclose; R. Ahlbrand, Nothing to Disclose; P. Horn, Nothing to Disclose; N. Richtand, **Part 1:** Bristol-Meyers Squibb, Gerson Lehrman Group, Sunovion Pahrmaceuticals, Sepracor Speaker Bureau, Otsuka Amercia Pahrmaceuticals, Schering-Plough Corp, Merck, Novartis, Ortho-McNeil Janssen Scientific Affairs, LLC, AstraZeneca Pharmaceuticals, Department of Veterans Affairs.

M161. Effects of Chronic Cariprazine Administration on Serotonin and Glutamate Receptor Subtypes

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Background: Accumulating evidence has implicated dysregulation of the serotonergic and glutamatergic systems in the pathophysiology of schizophrenia. In addition to their dopaminergic effects, atypical antipsychotics (AAs) modulate serotoninergic and glutamatergic neurotransmission which may contribute to their clinical efficacy, tolerability and long-term maintenance. Previous studies in rats have shown that long-term treatment with the AAs olanzapine, risperidone, quetiapine, and asenapine increases serotonin 5-HT_{1A} levels in the medial-prefrontal cortex (MPC) and dorsolateral frontal cortex (DFC) while decreasing serotonin 5-HT_{2A} levels in the same regions. Long-term treatment with AAs also altered the levels of ionotropic glutamate N-methyl-D-aspartic acid (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Olanzapine, risperidone, quetiapine, and asenapine all decreased NMDA receptor levels in the caudate-putamen (CP). Additionally, olanzapine and risperidone decreased NMDA receptor levels in hippocampal CA_1 and CA_3 regions; asenapine decreased NMDA receptor levels in the nucleus accumbens (NAc). Chronic administration of olanzapine, risperidone, and quetiapine increased AMPA receptor levels in the CP, while asenapine increased AMPA receptor levels in hippocampal CA_1 and CA_3regions. Cariprazine is a dopamine receptor D_3-preferring D_3/D_2 partial agonist antipsychotic candidate in development for the treatment of schizophrenia and bipolar disorder, and as adjunctive therapy for major depressive disorder. In earlier studies, cariprazine was shown to increase dopamine D_2 and D_4 receptor levels in select regions of rat forebrain similar to other AAs; however, cariprazine was unique in that it was the only drug that increased dopamine D_3 receptor levels in D_3-expressing brain regions. In this study, we examined the long-term effects of cariprazine on 5-HT_{1A}, 5-HT_{2A}, NMDA, and AMPA receptors in different regions of rat forebrain and compared these results to other AAs as determined in previous studies.
Methods: Male Sprague-Dawley rats initially weighing 200–225 g, were maintained in a controlled environment. Groups of 8 rats received control (1 ml/kg) or one of three doses of cariprazine (0.06, 0.2 and 0.6 mg/kg) administered by intraperitoneal (IP) injections for 28 d. At the end of treatment, rat brains were harvested and the following brain tissues were collected: MPC, DFC, medial CP (CP-M), lateral CP (CP-L), NAc, olfactory tubercle (OT), hippocampal regions CA_1 and CA_3, and entorhinal cortex (EC). Autoradiographic assays to determine 5-HT_{1A}, 5-HT_{2A}, NMDA, and AMPA receptor levels were performed using appropriate radioligands and blocking agents for the different receptors. Images from each autoradiographic assay were analyzed, the optical density of sampled regions was measured, and the amount of ligand bound within each area was calculated as nCi/mg tissue and converted into fmol bound/mg tissue. Two-way ANOVA evaluated overall changes across treatments and brain regions. Comparisons were considered significant at $P<.05$ in 2-tailed tests, with 8 subjects/group.
Results: Chronic cariprazine treatment (0.06, 0.2 and 0.6 mg/kg) dose-dependently and significantly increased 5-HT_{1A} receptor levels in hippocampal CA_1 (33%, 42% and 50%, respectively) and CA_3 (29%, 39% and 52%) regions. Cariprazine (0.2 and 0.6 mg/kg) increased 5-HT_{1A} receptor levels in MPC (41% and 61%) and DFC (42% and 61%). Cariprazine did not alter 5-HT_{2A} receptor levels in any of the brain regions examined. Cariprazine (0.2 and 0.6 mg/kg) significantly reduced NMDA receptor levels in NAc (−37% and −40%), CP-M (−34% and −38%), and CP-L (−35% and −38%), and in hippocampal CA_1 (−25% and −28%) and CA_3 (−23% and −30%) regions. Cariprazine (0.2 and 0.6 mg/kg) dose-dependently increased AMPA receptor binding in hippocampal CA_1 (31% and 47%) and CA_3 (34% and 50%)
Conclusions: Cariprazine treatment induced regional increases in 5-HT_{1A} receptor levels in the cerebral cortex similar to other AAs; unlike other AAs, cariprazine treatment was also associated with increased 5-HT_{1A} receptorlevels in hippocampal CA_1 and CA_3 regions. While other AAs decreased 5-HT_{2A} receptor levels in the cerebral cortex, cariprazine did not significantly affect 5-HT_{2A} receptor levels in any brain region tested. Cariprazine showed similar effects as other AAs on NMDA receptor levels in the CP and hippocampus, though like asenapine, cariprazine was also associated with decreased NMDA receptor levels in the NAc. Repeated cariprazine treatment induced elevation in AMPA receptor levels similar to asenapine, but not other AAs. These findings show that cariprazine displays effects on serotonin and glutamate receptor expression that are similar to other AAs but also shows distinct pharmacological effects, particularly in the hippocampal regions. The unique pharmacological effects of cariprazine may contribute to its clinical efficacy and tolerability profile.
Keywords: Autoradiography, Cariprazine, Serotonin, Glutamate, Schizophrenia, NMDA, AMPA
Disclosure: F. Tarazi, **Part 4:** Research grants from Forest, Novartis, Lundbeck and Shire; Y. Choi, Nothing to Disclose; N. Adham, **Part 1:** Employee of Forest Research Institute; B. Kiss, **Part 1:** Empolyee of Gedeon Richter Plc; I. Gyertyan, **Part 1:** Employee of Gedeon Richter Plc

M162. Effects of a Novel Simplified Acute Tryptophan Depletion (SATD) Vs. Acute Tryptophan Depletion (ATD Moja-De) Formulas on Serotonergic and Dopaminergic Content in C57BL/6J Mice

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Background: Several studies have used tryptophan-free amino acid mixtures to reveal the role of serotonin on several psychiatric disorders, including depression, anxiety, and schizophrenia. Our aim was to study the effects of an innovative tryptophan depletion

mixture on blood and brain serotonergic and dopaminergic metabolites in mice, in comparison with a control condition and a tryptophan-free mixture (ATD Moja-De). This new simplified acute tryptophan depletion formula (SATD) is based on ATD Moja-De, but is composed of only three large neutral amino acids (LNAA): Phenylalanine (PHE), Isoleucine (ILEU) and Leucine (LEU). These amino acids compete with tryptophan (TRP) for access into the brain as they are transported across the blood brain barrier by the same specific transporter (L-1) and thereby a tryptophan-free mixture of LNAA lowers serotonin synthesis.

Methods: To investigate the neurochemical consequences of SATD and ATD Moja-De administration, we treated adult male C57BL/6J mice from Jackson Laboratory with 2g/Kg body weight in deionized water. Mice were housed in groups of 6-8 and kept under a 12:12 light/dark cycle in a temperature-controlled room. Food-deprived animals were gavaged with either a balanced (BAL) formula containing all relevant LNAA (control condition), ATD Moja-De lacking TRP or the new SATD mixture in two doses spaced 30 min apart. 2.5 h after the first dose, when serotonergic metabolites are maximally depleted, mice were anesthetized with isoflurane and euthanized following the guidelines of the Animal Care and Use Committee at Duke University Medical Center. Blood was collected by cardiac puncture and brain regions (hippocampus, frontal cortex, amygdala, caudate putamen and nucleus accumbens) were dissected on ice using a mouse brain block and immediately frozen at -80°C until HPLC analysis. To quantitate tryptophan, monoamines and their metabolites, we used two RP-HPLC systems with electrochemical detection and C18 columns. A fresh standard curve was used every new day of analysis.

Results: Compared to control condition (BAL), ATD Moja-De mixture showed overall significantly decreased levels of TRP, 5-HIAA and 5-HT in blood and brain tissue. As hypothesized, SATD also significantly reduced serotonergic metabolite levels in blood and brain in line with ATD Moja-De results. SATD also reduced HVA levels in caudate but did not alter total DA levels or DOPAC in this or any other region.

Conclusions: We developed and assayed a new amino acid mixture containing a simplified formula lacking TRP which elicited a significant decrease in TRP and serotonergic metabolites in most of the regions we collected. We also observed an unexpected decrease in one dopaminergic metabolite in the caudate putamen but otherwise the DA system was unaffected. The similar efficacy of SATD and ATD Moja-De to deplete serotonergic content in the brain suggests that a simplified mixture of amino acids can compete with TRP at the blood brain barrier enough to significantly impair its transport. These results suggest that a simplified combination of amino acids can be used to examine the neurochemical underpinnings of some psychiatric diseases.

Keywords: serotonin, tryptophan depletion, mouse model

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M163. Patterns in the Shape of Maturational Trajectories across the Human Cortex and between the Sexes

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Background: The developmental trajectory of cortical thickness during childhood and adolescence varies across the brain. Previous work has classified cortical regions based on whether the relationship between age and thickness is best captured by linear, quadratic or cubic functions. When there is evidence for an "inverted U"-type curve, regions can also be differentiated based on the peak of these curves. Such criteria have demonstrated differences between typically developing male and female children, as well as alterations in neurospychiatric diseases including ADHD and childhood-onset schizophrenia. Hypothesis testing related to differences in the shape of maturational trajectories, both between brain regions and between study populations, is limited by two factors. **I**) It is not clear how to quantify differences in the shapes of growth curves, especially if they have been modeled by polynomial functions of different order (e.g. quadratic vs. cubic). **II**) These growth curves are highly dependent on the precise age-range sampled, which can lead to false inferences about key features such as the location of the peak of a maturational trajectory. Here we used a well-validated nonparametric local smoothing technique, penalized splines, to address these limitations. We demonstrate patterns of similarly-shaped maturational trajectories across the human cortex, as well as regional differences in the shape of these developmental curves between the sexes.

Methods: The study sample consisted of 858 longitudinally acquired structural MRI scans from 517 unique subjects (255 female, 262 male), representing 354 families and ranging in age from 5 to 30 years. All scans were acquired on the same 1.5T General Electric scanner in Bethesda, MD, using a Spoiled Gradient Echo sequence. Image processing was performed using the Montreal Neurological Institute's CIVET pipeline to automatically estimate thickness at ~80,000 vertices across the cortex. Statistical analysis was performed in R using the *vows* library (http://cran.r-project.org/). At every vertex, average thickness was estimated as a smooth function of age using semiparametric mixed models with standard parameters (10 knots; cubic B-spline functions; a second order derivate penalty; person- and family-level random effects). K-means clustering was applied to the set of estimated maturational trajectories for each cortical vertex, to explore patterns of coordinated maturation across the cortex. As the input to the clustering algorithm, the distance between two maturational trajectories (function estimates) was defined as the L^2 distance (square root of the integral of the squared difference) between their first derivatives. This distance was also used to determine for each vertex whether the shape of its maturational trajectory differed between the male and female samples, using a permutation procedure for hypothesis testing and correction for multiple comparisons.

Results: Clustering maturational curves of cortical thickness revealed new patterns of relatedness between cortical regions. Clusters respected *a priori* anatomical boundaries and homotopic relationships between symmetric interhemispheric regions, but also demonstrated significant relationships between brain regions whose maturational trajectories had not previously been demonstrated to be similar. Most of the differences between clusters were due to differences in the rate of thickness decline during adolescence, as opposed to the location of peaks. In fact, in contrast to previous work, only a minority of the brain (primarily within temporal lobes) demonstrated any period of increasing cortical thickness within this 5-30 year interval. Groups of vertices were identified whose trajectory shapes differed between the male and the female samples ($P < .05$, family-wise error corrected for

multiple comparisons). These differences were localized to regions including the cingulate and the temporal-parietal junction. We did not find statistical evidence for differences in the pattern of curve similarity across the cortex (i.e., the membership of the k-means clusters was not significantly different between men and women). However, preliminary work on a matched childhood-onset schizophrenia sample suggests that this pattern itself could be altered in this patient population.
Conclusions: Pattern classification of maturational trajectories reveals previously unknown differences between male and female neurodevelopment. We are able to address for the first time the question of which regions possess similarly-shaped growth curves across childhood, adolescence and early adulthood. The application of these methods to disease samples will enable the testing of novel hypotheses about neurodevelopmental pathology.
Keywords: Growth curves, Structural MRI, Sex differences, Penalized splines, Pattern classification
Disclosure: A. Alexander-Bloch, Nothing to Disclose; P. Reiss, Nothing to Disclose; N. Gogtay, Nothing to Disclose; J. Giedd, Nothing to Disclose.

T164. Drug Cue-induced Dopamine Release in Amygdala and Hippocampus: A High-resolution PET [18F]Fallypride Study in Cocaine Dependent Participants

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Background: Drug related cues are potent triggers for relapse in people with cocaine dependence. Dopamine release within a limbic network of striatum, amygdala and hippocampus has been implicated in animal studies, but in humans it has been possible to test the first region only. The objective here was to measure effects in the amygdala and hippocampus using high-resolution PET with [^{18}F]fallypride.
Methods: Twelve cocaine dependent volunteers (mean age: 39.6 ± 8.0; years of cocaine use: 15.9 ± 7.4) underwent two PET [^{18}F]fallypride scans with a Siemens HRRT camera, one with exposure to neutral cues and one with cocaine cues. [^{18}F]Fallypride non-displaceable binding potential (BP_{ND}) values were derived for five regions of interest (ROI) (ventral limbic striatum, associative striatum, sensorimotor striatum, amygdala, and hippocampus). Subjective responses to the cues were measured with visual analog scales and grouped using principal component analysis.
Results: Individual differences in the cue-induced craving factor predicted [^{18}F]fallypride responses in the ventral limbic ($r = 0.581$, $p = 0.048$), associative ($r = 0.589$, $p = 0.044$) and sensorimotor striatum ($r = 0.675$, $p = 0.016$); the greater the craving, the greater the [^{18}F]fallypride response. When participants were split into craving responders ($n = 6$) and non-responders ($n = 6$), drug cue exposure significantly decreased BP_{ND} values in the craving group in all five ROI (limbic striatum: $p = 0.019$, associative striatum: $p = 0.008$, sensorimotor striatum: $p = 0.004$, amygdala: $p = 0.040$, and right hippocampus: $p = 0.025$), but not in the non-responders.
Conclusions: To our knowledge this study provides the first evidence of drug cue-induced dopamine release in the amygdala and hippocampus in humans. The preferential induction of dopamine release among cue-responders suggests that these aspects of the limbic reward network might contribute to drug seeking behavior.
Keywords: addiction, craving, reward, striatum, limbic, conditioning
Disclosure: A. Fotros, Nothing to Disclose; K. Casey, Nothing to Disclose; K. Larcher, Nothing to Disclose; J. Verhaeghe, Nothing to Disclose; S. Cox, Nothing to Disclose; P. Gravel, Nothing to Disclose; A. Reader, Nothing to Disclose; A. Dagher, Nothing to Disclose; C. Benkelfat, Nothing to Disclose; M. Leyton, Nothing to Disclose.

M165. Lurasidone for Bipolar I Depression: Effects on Quality of Life and Functioning

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Background: Bipolar disorder is a chronic and often disabling illness with a lifetime prevalence of ~4.4% (Merikangas et al, Arch Gen Psychiatry 2007;64:543-52). Major depressive episodes occurring in bipolar I disorder are accompanied by markedly impaired ability to function (Keck et al, J Psych Practice 2008;14 [Suppl 2]:31-38). The goal of this secondary analysis was to evaluate the effect of lurasidone, both as monotherapy (PREVAIL 2) and adjunctive to lithium or valproate (PREVAIL 1), on quality of life and functioning in patients with bipolar I depression.
Methods: In both PREVAIL 1 and PREVAIL 2, subjects meeting DSM-IV-TR criteria for bipolar I depression, with or without rapid cycling, with a Montgomery-Asberg Depression Rating Scale (MADRS) score ≥ 20 and a Young Mania Rating Scale score ≤ 12, were randomized to 6 weeks of once-daily, double-blind treatment with either lurasidone or placebo. In PREVAIL 1, subjects received either lurasidone 20-120 mg/day (LUR20-120) or placebo (PBO), both adjunctive to stable treatment with either lithium (Li) or valproate (VPA). In PREVAIL 2, subjects received either lurasidone 20-60 mg/day (LUR20-60), lurasidone 80-120 mg/day (LUR80-120) or placebo (PBO). In both studies, the primary endpoint was change from baseline to Week 6 in the MADRS total score evaluated using a mixed model repeated measures (MMRM) analysis. Functioning was assessed in secondary analyses using the Sheehan Disability Scale (SDS) total and subscores. Quality of life (QoL) was assessed using the Quality of Life, Enjoyment, and Satisfaction scale short-form (Q-LES-Q-sf). SDS and Q-LES-Q-sf outcomes were evaluated using analysis of covariance (ANCOVA) with last observation carried forward (LOCF), and logistic regression.
Results: In PREVAIL 1, adjunctive treatment with LUR20-120 resulted in significantly greater reduction in MADRS total score at Week 6 compared with placebo (−17.1 vs. −13.5; $p = 0.005$). Lurasidone treatment also significantly improved function as demonstrated by an SDS total score change of −9.5 ($p = 0.012$) for LUR20-120 vs. − 7.0 for PBO (LOCF); and significant improvements on the SDS-social life subscore ($p = 0.003$), the SDS-family life/home responsibilities subscore ($p = 0.003$), and a non-significant trend towards improvement on the SDS-work/school subscore ($p = 0.068$). Treatment with LUR20-120 vs. PBO resulted in significant improvement at Week 6 on the Q-LES-Q-sf total score (+22.2 vs. +15.9; $p = 0.003$; LOCF), and a significantly higher proportion of subjects shifting from below normal to normal (≥ 52.3) Q-LES-Q-sf scores (64.3% vs. 44.1%; $p = 0.001$). In PREVAIL 2, lurasidone monotherapy significantly reduced MADRS total score at Week 6 for both the LUR20-60 group (−15.4; $p < 0.001$) and the LUR80-120 group (−15.4; $p < 0.001$) vs. PBO (−10.7). Both lurasidone groups also showed significantly improved function, with greater Week 6 reduction in the SDS total score for both the LUR20-60 group (−9.5; $p = 0.003$) and the LUR80-120 group (−9.8; $p < 0.001$) vs. PBO (−6.3), and greater improvements for all SDS subscores. Significant improvement was observed at Week 6 on the Q-LES-Q-sf total score for both the LUR20-60 group (+19.3; $p = 0.001$) and the LUR80-120 group (+19.8; $p = 0.001$) vs. PBO (+12.8), and a significantly higher proportion of subjects shifted from below normal to normal Q-LES-Q-sf scores for the LUR20-60 group (55.3%; $p < 0.001$) and the LUR80-120 group (54.2%; $p < 0.001$) vs. PBO (32.9%).
Conclusions: In this analysis of data from two 6 week studies, treatment of bipolar I depression with lurasidone, whether monotherapy or adjunctive to Li or VPA, was associated with

significant improvement in quality of life and functioning as measured by patient-rated scales.
Keywords: bipolar depression, lurasidone, functioning, quality of life
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M166. Decreased Neuropeptide Y and Altered Neuropeptide Y - corticotropin Releasing Hormone Balance in Cerebrospinal Fluid in Remitted Bipolar Patients Can Predict Future Suicide Attempts

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Background: The life-time prevalence of bipolar disorder (BP) is 1.5-3% and the consequences for the individual and society are dire; the attempted and accomplished suicide rates are about 50 and 15-20%, respectively (Baldessarini RJ et al 2006; Tidemalm D et al 2008). Previous suicide attempts and psychiatric diagnoses (eg, BP, alcoholism, schizophrenia) indicate a markedly higher suicide risk. However, the neurobiology and biomarkers have not been identified. Neuropeptide Y (NPY), a highly evolutionary conserved peptide is widely distributed in brain regions and has myriad effects in the CNS (Wu G et al 2011). We and others have shown that NPY expression is decreased in environmental and genetic animal models of depression and PTSD, in cerebrospinal fluid (CSF) from PTSD patients and treatment resistant MDD patients, and in postmortem brains from BP patients and subjects who committed suicide. Further, all so far tested antidepressant treatments increase NPY in brain and CSF (Björnebekk A et al 2010; Caberlotto L and Hurd Y 1999; Cohen H et al 2012; Heilig M et al 2004; Husum H and Mathé AA 2002; Jiménez-Vasquez PA et al 2000, 2001; Mathé AA et al 1990, 1998, 2007; Nikisch G et al 2005, 2011; Sah R et al 2009; Stenfors C et al 1989; Widdowson PS et al 1992). In contrast to the anxiolytic effects of NPY, corticotropin releasing hormone (CRH) is an anxiogenic peptide that plays a role in stress disorders. NPY and CRH have opposing effects in regulation of emotionality and NPY is likely an endogenous buffer against the stressor-induced CRH release (Heilig M et al 1994; Thorsell A 2010). In view of the above we measured NPY and CRH in the CSF from euthymic BP patients and analyzed the results in relation to past history (eg, suicide attempts) and clinical outcome (eg, suicide attempts after collection of CSF).
Methods: The project was approved by the IRB, Karolinska Institutet and patients recruited from the St Göran's Bipolar Project (Rydén E et al 2009). The clinical assessment instrument was the Affective Disorder Evaluation used in the STEP-BD project (Sachs GS et al 2003). The somatic trait anxiety, psychic trait anxiety, and stress susceptibility were assessed with the Swedish universities Scales of Personality. Euthymia was defined as MADRSmedication was allowed at the time of lumbar puncture (LP) which was done when the participants were in a stable euthymic mood. NPY and CRH like-immunoreactivities (LI) were measured in CSF (Nikisch G et al 2005). A one-year follow-up was carried out and suicide attempts, designated "prospective suicide attempts", that occurred in the interval between the LP and follow-up time point recorded.
Results: 120 BP patients were included. Of these, 52 had a history of suicide attempts. At the one year follow-up, 8/120 had attempted suicide after the LP. Six of these 8 patients had also attempted suicide prior to the LP. The most salient results (119/120, one CRH sample lost): NPY-LI in CSF and suicide *No previous attempts: $n = 68$, $M = 47.50$, $SD = 35.18$, vs previous attempts: $n = 51$, $M = 34.65$, $SD = 26.38$; T-test: $t = 2.186$, $p = 0.031$. *No prospective attempts: $n = 111$, $M = 43.93$, $SD = 32.34$, vs prospective attempts: $n = 8$, $M = 15.08$, $SD = 13.36$; Mann-Whitney: $U = 145$, $p = 0.002$. *NPY-LI was lower in patients with previous suicide attempts compared to patients without previous attempts. There was a negative correlation between NPY-LI and the number of previous attempts ($rho = -0.224$, $n = 120$, $p = 0.014$). *NPY-LI was lower in patients with prospective suicide attempts compared to patients without prospective attempts. Finally, the NPY-LI reduction in prospective suicide attempters was significant also when compared

to the patients with previous attempts who did not repeat suicide attempt during the follow-up period (Mann-Whitney: U = 78, p = 0.01). CRH-LI in CSF and suicide No significant differences were found. NPY/CRH and suicide *No previous attempts: n = 68, M = 5.24, SD = 5.03, vs previous attempts: n = 51, M = 2.98, SD = 2.25; T-test: t = 2.993, p = 0.001. *No prospective attempts: n = 111, M = 4.47, SD = 4.28, vs prospective attempts: n = 8, M = 1.53, SD = 1.29; Mann-Whitney: U = 170, p = 0.004. NPY and temperament scales There was a negative correlation between NPY-LI and psychic trait anxiety (rho = −0.209, n = 114, p = 0.025). NPY/CRH and suicide *No previous attempts: n = 68, M = 5.24, SD = 5.03, vs previous attempts: n = 51, M = 2.98, SD = 2.25; T-test: t = 2.993, p = 0.001. *No prospective attempts: n = 111, M = 4.47, SD = 4.28, vs prospective attempts: n = 8, M = 1.53, SD = 1.29; Mann-Whitney: U = 170, p = 0.004. NPY and temperament scales There was a negative correlation between NPY-LI and psychic trait anxiety (rho = −0.209, n = 114, p = 0.025).

Conclusions: Our study shows for the first time that NPY levels in the CSF are reduced in euthymic BP patients with a history of previous suicide attempts compared to BP patients without previous attempts. Moreover, patients who attempted suicide during a one year follow-up had a larger NPYreduction compared both to patients without/with previous suicide attempts. These results are in line with the hypothesis about the central role of NPY in affective disorders/ emotional dysregulation and indicate that NPY in CSF can be a predictor of risk for suicide attempts. The findings also support our hypothesis that increasing NPY system activity with epigenetic drugs that enhance NPY expression, or with exogenousNPY, or non-peptidergic NPY specific receptor agonists should open new avenues to treat affective disorders.

Keywords: bipolar disorder, suicide, biomarkers, neuropetide Y, CRH,

Disclosure: A. Mathé, Nothing to Disclose; J. Sandberg, Nothing to Disclose; M. Landén, Nothing to Disclose.

M167. Abnormal Threat Detection in AHI1 Mutant Mice: Translational Relevance to Schizophrenia and Autism

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Background: The Abelson helper integration site (*AHI1*) gene plays a pivotal role in brain development. Loss of function mutations in *AHI1* cause Joubert Syndrome, a severe neurodevelopmental disorder. Studies by our group, replicated by several others, have demonstrated association of single nucleotide polymorphisms in *AHI1* with susceptibility to schizophrenia. Furthermore, we have demonstrated altered expression of *AHI1* in lymphoblasts from early onset schizophrenia patients. Association of *AHI1* with autism has also been reported. To elucidate the mechanism whereby alteration in *AHI1* expression may be implicated in the pathogenesis of endophenotypes related to severe neuropsychiatric disorders, we have studied mice heterozygous ($Ahi1^{+/-}$) for an *Ahi1* knockout mutation as compared to wild type ($Ahi1^{+/+}$) littermates. We employed behavioral paradigms that model different aspects of schizophrenia, pharmacological challenges, histological studies and resting state functional connectivity imaging (rsfMRI).

Methods: We studied the association between reduction in *Ahi1* expression and schizophrenia-like phenotypes by comparing the performance of $Ahi1^{+/-}$and WT $Ahi1^{+/+}$ mice on behavioral tests that model cognitive (Morris water maze, novel object recognition), negative (forced swim test, novelty suppressed feeding test), and positive (prepulse inhibition, MK-801 induced hyperlocomotion) aspects of schizophrenia as well as tests reflecting anxiety such as elevated plus maze (EPMT), light dark box (LDBT) and open field (OFT) tests. Serum cortisol and core body temperature were measured following behavioral and pharmacological experiments. We sought structural abnormalities in the brains *of* $Ahi1^{+/-}$ mice by light microscopy and have begun to dissect neural circuits through the use of functional connectivity imaging during resting state (rsfMRI). This methodology enables generation of connectivity maps between predefined regions of interest (ROIs).

Results: RT-PCR revealed reduced expression of *Ahi1* mRNA in $Ahi1^{+/-}$ compared to $Ahi1^{+/+}$ mice. Western blots showed significantly lower Ahi1 protein levels in $Ahi1^{+/-}$ newborns that were not present in adult mice. Behavioral testing did not reveal abnormalities of $Ahi1^{+/-}$ mice on measures reflecting cognitive function and positive or negative schizophrenia symptoms. On the other hand significantly lower levels of situational anxiety in $Ahi1^{+/-}$ mice were a consistent finding. In the OFT, $Ahi1^{+/-}$ mice spent more time in the arena center (p = 0.02) compared to $Ahi1^{+/+}$ mice; in the EPMT more time in the maze open arms (p = 0.006) and in the LDT, more time in the open lightened zone (p = 0.02). Serum cortisol levels and core body temperature, two highly consistent endophenotypes relevant to the mammalian stress response, were significantly lower in $Ahi1^{+/-}$ than $Ahi1^{+/+}$ mice after exposure to situationaly provoked anxiety in the EPM test (p = 0.02 and p = 0.03, respectively). To establish whether the ability of $Ahi1^{+/-}$ mice to "feel" anxiety is different from that of WT mice blood we measured serum cortisol level after anxiogenic challenge with caffeine (80 mg/kg) and acoustic startle (average of 30 trials amplitude). Cortisol levels were not different in $Ahi1^{+/-}$ and $Ahi1^{+/+}$ mice. No appreciable abnormalities in the volume of the cerebrum, the cerebellum or the olfactory bulb were observed in the brains of $Ahi1^{+/-}$ mice in adulthood according to light microscopy and structural T2-weighted MRI. Examination of brain structure using H&E staining revealed normal cytoarchitecture of the prefrontal cortex, the basal ganglia and the hippocampus in $Ahi1^{+/-}$ mice. The volume of the amygdala, a structure that has been consistently implicated in the process of threat detection was not reduced. However, preliminary rsfMRI data indicate that significant connections in $Ahi1^{+/+}$ mice, such as those involving the neocortex, cerebellum and amygdala are diminished in $Ahi1^{+/-}$ mice. The data also suggest loss of symmetry in $Ahi1^{+/-}$ mice, most prominent for connections involving the primary somatosensory cortices and various subcortical structures.

Conclusions: Our findings indicate that reduced expression of the *Ahi1* gene in mice is associated with a decrease in perception of threatening situations even though the $Ahi1^{+/-}$ mice manifest a similar degree of anxiety to $Ahi1^{+/+}$ mice, reflected in serum cortisol level, on exposure to caffeine challenge and acoustic startle. Reduced threat detection, as manifested by $Ahi1^{+/-}$ mice, may arise from reduced connectivity between the amygdala and other forebrain areas, including the cortex; this finding has been reported in schizophrenia and autism. The present findings suggest that neurodevelopmental abnormalities associated with reduced expression of *AHI1* in the fetus and newborn and correspondingly lower Ahi1 protein levels may have significant functional consequences in the adult. Such a mechanism is consistent with currently postulated neurodevelopmental theories of schizophrenia. The present data are a translational demonstration of the feasibility of such a model in relation to the role of *AHI1*, a gene replicably associated with schizophrenia, in the pathogenesis of the disorder. *Supported in part by a grant from the Israel Science Foundation (BL) and by a Young Clinician Investigator Award from Hadassah Medical Organisation (AL)Amit Lotan and Tzuri Lifschytz contributed equally to this work.*

Keywords: schizophrenia, ahi1, neurodevelopment, threat detection, amygdala

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M168. Activation of Noradrenergic Locus Coeruleus Neurons Promotes Anxiety-like Behaviors

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Background: The locus coeruleus (LC) and its projections are the primary source of norepinephrine for the mammalian forebrain. The LC is a target for corticotropin-releasing factor (CRF), opioid neuropeptide containing neurons, and is highly enriched with all four opioid receptor types. LC tonic firing increases during stress and this increase is thought to be controlled by opioid and CRF activity.
Methods: Therefore, we asked if specific modulation of LC neuronal firing could lead to anxiety-like and aversive behaviors. Genetically targeting channelrhodopsin-2 to only LC noradrenergic (LC/NA) neurons, we used optogenetic light stimulation to specifically increase LC/NA neuronal activity. We also investigated the role of beta-adrenergic Gs-signalling in negative affective behaviors including aversion and anxiety using optogenetics. Utilizing previously generated (Airan et al, Nature 2009) chimeric rhodopsin and beta2-AR receptors (OptoXRs) we pharmacologically characterized and compared the properties of light-induced optoXRs to agonist-induced beta2-AR signalling in terms of cAMP generation and the phosphorylation of ERK MAPK signalling *in vitro*.
Results: We report an increase in tyrosine hydroxylase and c-fos co-immunoreactivity in the LC following light stimulation, as well as consistent firing of LC, NA neurons following repeated 5hz light stimulation *in vitro* and *in vivo*. We then examined the effect of increasing tonic LC/NA firing on anxiety- and aversion-like behaviors using a conditioned place aversion paradigm. We found that increasing tonic firing of LC/NA neurons in a context dependent manner, consistent with CRF release in the LC, induces a subsequent aversion to the stimulated context. Furthermore, increased LC/NA firing results in decreased open arm time in the elevated zero maze, and time in the center of the open field test (OFT), both assays for anxiety-like behavior. To assess the role of putative NA post-synaptic beta-adrenergic Gs-signalling in aversion and anxiety, we used a real-time place conditioning behavioral paradigm. When we selectively expressed Beta2-optoXRs in the BLA of mice and allowed for real-time optical stimulation of beta2-optoXRs, mice displayed a robust aversive response to the conditioning chamber as compared to control mice injected with empty vector but also receiving light stimulation. Further, we also used the OFT and found that when beta2-optoXRs are stimulated in the BLA, mice display a dramatic reduction in the amount of time spent in the center of the arena with no significant changes in locomotor behavior. We also investigated the central amygdala inputs into the LC that regulate its firing, and the NA neuronal projections back to the amygdala in these behaviors.
Conclusions: These data suggest that increasing the firing rate of LC/NA neurons can modulate affective behaviors including aversion and anxiety. These data support also the conclusion that activation of Gs signalling in excitatory neurons of the BLA is sufficient to produce avoidance and anxiogenic-like behavioral effects. Together these results suggest that noradrenergic locus coerlueus tone is important for anxiety and aversive behavioral states.
Keywords: norepinephrine, GPCR, CRF, stress, anxiety
Disclosure: J. McCall, Nothing to Disclose; E. Siuda, Nothing to Disclose; C. Ford, Nothing to Disclose; M. Bruchas, Nothing to Disclose.

M169. Solanezumab Phase 3 Results: Testing the Amyloid Hypothesis

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Background: Solanezumab is a humanized monoclonal antibody developed for the treatment of Alzheimer's disease (AD). It binds to the mid-domain of soluble amyloid beta (A-beta) peptide but not to deposited amyloid plaques.
Methods: Patients were enrolled in two Phase 3 trials (EXPEDITION and EXPEDITION 2) between May, 2009, and December, 2010. Participants had mild to moderate AD, determined using National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criter, and Mini-Mental State Exam scores of 16-26. Participants were randomized to 400 mg solanezumab IV or placebo once every 4 weeks for 80 weeks. The Alzheimer's Disease Assessment Scale - Cognitve subscale (ADAS-Cog), the Alzheimer's Disease Cooperative Study - Activities of Daily Living (ADCS-ADL) scale and the Clinical Dementia Rating - Sum of Boxes were assessed along with other measures of clinical efficacy. All participants underwent magnetic resonance imaging at baseline and at Weeks 12, 28, 52, and 80. A subset of participants underwent florbetapir PET imaging or lumbar pncture at baseline and endpoint.
Results: A total of 2052 participants were randomized. The fficacy endpoint findings, biomarkers and safety findings will be presented and discussed for the intent-to-treat population and relevant subgroups.
Conclusions: The phase 3 results will provide important efficacy and safety data for solanezumab, as relates to its use in patients with mild and moderate AD; these data will help to advance the understanding of the amyloid hypotheses of Alzheimer's disease.
Keywords: Alzheimer's disease, solanezumab, amyloid, biomarkers.
Disclosure: R. Mohs, **Part 1:** Richard C. Mohs is a full time employee and stockholder of Eli Lilly and Company; E. Siemers, **Part 1:** Eric Siemers is a full time employee and stockholder of Eli Lilly and Company; C. Carlson, **Part 1:** Christopher Carlson is a full time employee and stockholder of Eli Lilly and Company; W. Estergard, **Part 1:** Wahiba Estergard is a full time employee and stockholder of Eli Lilly and Company; K. Sundell, **Part 1:** Karen Sundell is a full time employee and stockholder of Eli Lilly and Company; D. Henley, **Part 1:** David Henley is a full time employee and stockholder of Eli Lilly and Company; J. Eads, **Part 1:** Jennifer Eads is a full time employee and stockholder of Eli Lilly and Company.; C. Sexton, **Part 1:** Cora Sexton is a full time employee and stockholder of Eli Lilly and Company; R. Dean, **Part 1:** Robert A. Dean is a full time employee and stockholder of Eli Lilly and Company; B. Willis, **Part 1:** Brian Willis is a full time employee and stockholder of Eli Lilly and Company; R. DeMattos, **Part 1:** Ron DeMattos is a full time employee and stockholder of Eli Lilly and Company.

M170. Chronic Ethanol Upregulates Toll-like Receptors Inc reasing Neuroinflammation and Neurodegeneration Mimicking Human Alcoholic Brain

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Background: Toll-like receptors (TLRs) signaling induces proinflammatory genes related to brain function, neuroinflammation and neurodegeneration. We have previously found alcohol to

induce neuroinflammation prompting studies of ethanol on TLR2, TLR3 and TLR4 receptors and their endogenous agonist, HMGB1.
Methods: Immunohistochemistry, western blot, ELISA and RTPCR were used to assess levels of TLR and HMGB1 in rat brain slices, mouse brain and human post-mortem brain.
Results: Human postmortem alcoholic frontal cortex has increased levels of TLR2, TLR3 and TLR4 + IR positive immunoreactive (+ IR) cells, compared to human moderate drinking control brain. Using a model of alcoholic binge drinking (5 g/kg, i.g., daily for 10 days) in C57BL/6 mice, we found increased mRNA and + IR for TLR2, TLR3 and TLR4 as well as the agonist HMGB1. In rat brain slice cultures ethanol increased HMGB1 levels in the media as well as increasing IL-1 β mRNA and protein. Neutralizing anti-bodies to HMGB1 and siRNA to HMGB1 or TLR4 block ethanol induction of IL-1β. These studies support the hypothesis that ethanol increases HMGB1/TLR proinflammatory signaling in brain. To evaluate the impact of ethanol increased HMGB1-TLR expression polyinosine-polycytidylic acid (poly I:C), a synthetic dsRNA TLR3 agonist, was used to activate innate immune signaling in mice. Ethanol pretreatment caused modest increases in mRNA and protein levels of TNFa, MCP-1 and IL-6 in brain, increased NADPH oxidase (NOX) $gp91^{phox}$ and production of O_2^- and O_2^- -derived oxidants, that were amplified by PolyIC and persisted for long periods of time.
Conclusions: These studies suggest alcohol increases brain HMGB1/TLR signaling in brain leading to persistent proinflammatory gene induction.
Keywords: toll-like receptors, ethanol, poly I:C, cytokines, neurodegeneration
Disclosure: F. Crews, Nothing to Disclose; L. Qin, Nothing to Disclose; R. Vetreno, Nothing to Disclose.

M171. MRS GABA and Glutamate Abnormalities in the Superior Temporal Gyrus and Their Association with Gamma Band Oscillation Abnormalities in Schizotypal Personality Disorder and Schizophrenia

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Background: Many studies from our lab and from others indicate the superior temporal gyrus (STG) shows volumetric reductions in schizotypal personality disorder (SPD) and in schizophrenia (SZ), reductions that in first episode SZ progress after onset. Moreover, P300 and mismatch negativity (MMN) ERPs show reductions in SZ that correlate highly with the extent of sMRI volumetric abnormalities and, in the case of MMN, show concomitant progression of abnormalities in first episode SZ. Data also indicate the gamma band oscillation (GBO, ~40 Hz) elicited by the auditory steady state response paradigm (ASSR) has a prominent origin in the STG. Despite this extensive body of data about SZ and SZ spectrum abnormalities there have been, to our knowledge, no magnetic resonance spectroscopy (MRS) studies in SPD and SZ of STG voxels measuring GABA and glutamate (Glu) metabolites, the neurotransmitters used by inhibitory neurons and pyramidal neurons whose interaction is responsible for the GBO. We here report SZ and SPD subjects have reductions of left STG GABA concentrations there are inversely correlated with GBO (less reduction, better GBO as measured by the Phase Locking Factor, PLF) and Glu increases that are directly correlated with GBO (higher Glu, worse GBO PLF).
Methods: To determine the feasibility and promise of MRS in these disorders, four neuroleptic-naïve SPD subjects, six chronic SZ, and four age- and PSES-matched healthy controls (HC) (all subjects male, ages 41-54) were recruited for this study from a well-characterized cohort of patients where full DSM-IV criteria were met for SPD and SZ. The following protocol was then acquired in the left and right STG using a voxel size of 20 x 30 x 20 mm (12 mL). Conventional PRESS, J-resolved PRESS, MEGA-PRESS, and 2D COSY acquisitions were used. PRESS data was analyzed using LCmodel. Glu quantification was undertaken on the peaks at 2.35 and Glx, a combination of Glu and Gln, at 3.75 ppm. Similarly GABA at 3.0 ppm as well as Glx at 3.7 and 2.3 ppm were quantified. 2D COSY data was post-processed using Felix 2007 where crosspeak volumes were measured including location (F2, F1 in ppm), amplitude, and volume normalized to the Cr crosspeak amplitude and volume, respectively.
Results: The ASSR data from 4 male DSM-IV SPD and 4 male age-matched HC showed a left GBO ASSR PLF deficit to 40 Hz stimulation. The GBO PLF was highly correlated with the Left STG MRS data in both SPD and HC subjects. The PLF decreased with increasing Left STG Glu ($rho = -0.98$, $p < 0.001$) and increased with increasing GABA ($rho = 0.89$, $p < 0.01$). This is supportive of our model of dysfunction based on GABA disinhibition and the resulting increased glutamatergic excitation. Findings were similar in the medicated chronic SZ, which had a finer grain topographic analysis. The ASSR PLF was measured at 58 electrodes in 6 chronic SZ and 4 HC and then, for analysis, collapsed into regions of left and right Temporal, ParaCentral and Lateral based on a larger sample (24 chronic SZ, 24 HC). In this larger sample the maximum SZ PLF deficits were highly left-lateralized There was a strong association between the Left temporal 40 Hz GBO ASSR in chronic SZ and the LC Model Glutamate and MEGA-PRESS GABA concentrations in the Left STG. The predicted association for Left STG MRS Glu values and the Left Temporal and Left ParaCentral ASSR PLF for SZ and HC was observed in in significant inverse (negative) correlation ($rho = -.66$, $p < 0.019$). Pearson r values were also significant. The predicted direct (positive) and significant correlation between Left STG GABA and the ASSR PLF in both Left Temporal and ParaCentral electrodes for SZ and HC was found ($rho = 0.55$, $p = 0.049$). We note the dispersion of the SZ GABA values and of the ParaCentral PLF allowed a within SZ group measurement without the distortion of a "ceiling effect" for Glu (where all values are high) and a "floor effect" for the PLF (all values are low). Indeed within the SZ group, Left STG MRS GABA and the Left Para-central PLF values are highly correlated, $rho = 0.771$, $p = 0.036$.
Conclusions: These data support our hypotheses about associations between ASSR and MRS values in SZ, an association also found in the SPD subjects, who show a genetic relationship to SZ. Of note, in the never neuroleptic-medicated SPD, ASSR and MRS STG values paralleled our findings in the medicated SZ, suggesting the SZ data were not artifacts of medication. These data are consistent with our prediction, based on our GABA-Glutamate imbalance hypothesis, that both SPD and SZ would show a reduced ASSR PLF that would be associated with both a reduced GABA and increased Glu, a finding compatible with pyramidal cell disinhibition and GBO disruption.
Keywords: superior temporal gyrus, magnetic resonance spectroscopy, gamma oscillation, GABA, Glutamate.
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M172. Mutations in the X-linked Endosomal Alkali Cation/proton Exchanger 6 - a New Genetic Model to Study the Neurodevelopmental Biology of Severe Autism

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Background: Behavioral therapy is recommended as first line treatment for autism, yet for a significant subset of patients, this

approach is ineffective or only partially effective. There is a critical need to identify neurodevelopmental mechanisms that cause "difficult-to-treat" autism in order to develop new treatments. Genetic mutations that are associated with severe autism-related disorders provide an important opportunity to study these neurodevelopmental mechanisms. In recent years, mutations in the endosomal Na+/H+ exchanger 6 (NHE6) (Gilfillan et al., 2008; Garbern et al., 2010) and related NHE9 (Morrow et al., 2008) have been implicated in severe autism-related disorders. Mutations in NHE6 appear to be among the most common forms of X-linked developmental brain disorders (Tarpey et al., 2009). Variants in NHE9 have also been correlated with attention-deficit/hyperactivity disorder. In this study, we investigate the cellular and neurodevelopmental abnormalities that result from NHE6 mutations. We further investigate the association of NHE6 with autism symptoms.

Methods: Our study involves several interdisciplinary approaches: First, we have recruited the largest group of families to date with children who have NHE6 mutations. Standardized neuropsychiatric assessments have been conducted including assessment of autism-related symptoms. We have established induced pluripotent stem cells (iPSCs) from a subset of affected and unaffected family members. Second, we have established an NHE6-null mouse line to investigate cellular and neurodevelopmental mechanisms. We have conducted live-fluorescent imaging of endosomal pH and slice electrophysiology to investigate cellular and circuit defects in these mice. Third, we have conducted a re-analysis of publically available post-mortem data to identify gene expression changes in NHE6 and NHE9 in autism brains.

Results: While our clinical dataset reflects the largest sample of patients with NHE6 mutations to date, the sample size remains small ($n = 9$ families, 10 children total). Nonetheless, we have been able to make several interesting observations relevant to severe autism. All children are non-verbal. Using the Social Communication Questionnaire, 50% meet screening criteria for Autism. An overlapping 40% were given a prior diagnosis of Angelman syndrome. 40% of parents report at least one profound regression. Medical co-morbidity is high: all children have epilepsy and two children had Nissen fundoplication to treat gastro-esophageal reflux. We also find a surprisingly high rate of *de novo* mutation. Using our new antisera to NHE6, we discover that NHE6 is localized to major, long-range fiber tracts *in vivo*. NHE6 mutant neurons show defects in axonal growth and defects in pH in the endosome compartment. Synaptic field strength in hippocampal slice preparations is reduced between 20-30% in mutant mice ($p < 0.01$) as are measures of pre-synaptic fiber volley (reduced by 10-20%, $p < 0.05$) consistent with abnormalities in axonal development. Finally, in our studies in autism post-mortem brain, through re-analysis of publically available data (Voineagu et al., 2011), we confirm that there is a statistically significant increase in the expression of NHE9 in cortical tissue derived from autism brains as compared to control (1.31 fold change, $p < 0.003$) (previously shown by Voineague et al., 2011; Garbett et al., 2012). We also discover a statistically significant, and not previously reported, decrease in NHE6 gene expression in autism brains (0.81 fold change, $p < 0.02$). Changes in expression of NHE6 and NHE9 genes are highly correlated with decreases in synapse function genes (correlation = −0.62, p = 1.6e-07 for NHE9; correlation = 0.89, p = 3e-20 for NHE6). Interestingly, gene network analysis on genes with expression differences highlighted genes involved in synapse ($p = 1.7e-7$), and new associations with vesicle biology genes ($p = 5.6e-7$), protein localization genes ($p = 6.5e-8$), and genes involved in neuron projections ($p = 6.4e-5$). As brains in this study were derived from participants with more severe autism (data not shown), taken together our data from postmortem studies and from studies of endosomal NHE6 support an important role for protein sorting, endosome/vesicle biology and axon development in severe forms of autism.

Conclusions: Our results provide strong support for an association between NHE6 and NHE9 and severe forms of autism. Studies of NHE6 mutations provide a new and important opportunity to dissect abnormalities in long-range circuitry related to severe autism and regressions. In ongoing work, we will determine the mechanisms by which NHE6 regulation of endosomal pH governs axonal development. Also, we are working to identify cellular defects in human neurons derived from iPSCs with NHE6 mutations. These results are expected to have a positive impact because they will provide a strong framework for the development of treatment strategies designed to repair circuit defects and/or to prevent regression in difficult-to-treat autism patients. This work was supported by a grant from the Simons Foundation and the Nancy Lurie Marks Foundation.

Keywords: neurodevelopment autism genetics electrophysiology endosomes

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M173. Resting Functional Connectivity of the Nucleus Accumbens in Apathetic Late-life Depression

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Background: The geriatric psychiatry literature is replete with descriptions of apathy syndromes of depression, including "masked depression" and "depression without sadness". Apathy affects 19-88% of depressed non-demented persons, is most prevalent in late onset depression, and is associated with disability and poor treatment response. Little is known about the neurobiology of apathy of depression and most is based on studies of degenerative disorders and stroke. Akinetic mutism, an extreme form of apathy, has been described in a variety of conditions involving the ventral striatum, ventral globus pallidus, medial thalamus and the dorsal ACC. This study conceptualized apathy of late-life depression as the behavioral expression of abnormal functional connectivity (FC) of the nucleus accumbens (NAcc) with structures related to mood regulation. This view was based on apathy studies on degenerative disorders and on the neurobiology of depression, which postulate abnormal interactions among the reward structures and subcortical and prefrontal regions. Accordingly, we compared resting FC of the NAcc among apathetic and non-apathetic depressed elders and normal elderly subjects.

Methods: We studied 26 subjects (≥ 60). Of these, 16 met had unipolar major depression (SCID/DSM-IV) and 10 had no psychiatric disorders. All signed consent approved by the IRBs of Weill-Cornell and of Nathan Kline Institute. Cognitive impairment was rated with the Mini Mental State Examination (MMSE), Hopkins Verbal Learning, Stroop Color Word and Trails A and B. Apathy was quantified with the Apathy Evaluation Scale (AES). The depressed group was divided into apathetic (AES $>$ 36.5, $N = 7$) and non-apathetic (AES < 36.5, $N = 9$) subjects. Depressed subjects were scanned (1.5T; EPI; TR = 2000 ms, a TE = 50 ms, flip-angle = 90 degrees, matrix = 64x64, FOV = 224 mm, 22 5 mm slices, no gap) after a 2-week psychotropic washout and controls were psychotropic free. MPRAGEs were segmented using FSL's FAST software, and normalized to MNI152 space. We used the FSL FLIRT program to transform resting-state data into MNI152 space using a 12 DOF linear affine transformation. Images were thresholded using clusters with $z > 2.3$ and a significance threshold of $p < .05$.

Results: There were no significant differences in education or cognitive performance between apathetic and non-apathetic depressed and normal subjects. However, depressed subjects had

greater depression (MADRS: $z = 4.2$, $p < .0001$) and disability (WHODAS: $z = 3.7$, $p < .0001$) than normal subjects. Left NAcc Seed: Apathetic depressed patients had lower FC with the right amygdala, right putamen, left caudate, and bilateral thalamus than non-apathetic depressed patients. Apathetic patients have higher FC with left dorsomedial PFC and left dorsal ACC. In comparisons with control subjects, apathetic depressed patients had lower FC in the right amygdala, bilateral putamen, bilateral globus pallidus, and increased FC with the left hippocampus, left frontal regions (superior frontal, OFC). Non-apathetic depressed patients had lower FC with the left dorsal ACC and higher with the inferior frontal gyrus.
Right NAcc Seed: Apathetic depressed patients had lower FC with right amygdala, right putamen, left caudate, bilateral thalamus, and left dorsomedial PFC to relative to non-apathetic patients. Apathetic depressed patients also had higher FC in the dorsomedial PFC and the left dorsal ACC compared to non-apathetic depressed patients. Apathetic depressed patients showed lower FC with the putamen and globus pallidus bilaterally. Non-apathetic depressed patients had lower FC with the OFC bilaterally.
Conclusions: Resting FC of the NAcc distinguished older patients with apathy and major depression from depressed patients without apathy and from normal older subjects. Apathetic depressed patients had low FC of the NAcc with the amygdala, caudate, putamen and thalamus and increased FC with the ventromedial PFC and dorsal ACC. Resting FC abnormalities of the NAcc were more pronounced and included more structures related to motivational and executive functions in apathetic than non-apathetic depressives. We had proposed that disruption of white matter connecting frontolimbic and frontostriatal regions predisposes late life depression. This view was based on the current understanding of the neuroanatomical substrate underlying mood regulation, which implicates a dysfunction in a ventral system (NAcc, ventral ACC, ventrolateral prefrontal cortex, amygdala, and insula) and a dorsal system (dorsal anterior cingulate, prefrontal cortex) leading to abnormalities in emotional perception and in effortful regulation of emotions, and executive dysfunction. The FC abnormalities noted in depressed patients are consistent with this conceptualization. FC abnormalities of non-apathetic depressed patients of this sample may disrupt mood regulation and contribute to depressive symptoms and signs. To our knowledge this is the first study to identify abnormalities in resting FC in ventral striatum structures distinguishing apathetic depressed older patients from non-apathetic depressed patients and non-depressed subjects. However, these findings are based on a small number of subjects and require replication.
Keywords: Apathetic Geriatric Depression, Nucleus Accumbens, Functional Connectivity
Disclosure: G. Alexopoulos, **Part 1:** Avanir, Hoffman-LaRoche, Novartis, Otsuka, Pfizer, Sunovion, **Part 2:** Astra Zeneca, BMS, Lilly, Merck, **Part 3:** Forest, **Part 4:** Forest; G. Yuen, Nothing to Disclose; M. Hoptman, Nothing to Disclose; F. Gunning-Dixon, Nothing to Disclose.

M174. The Ankyrin 3 (ANK3) Bipolar Disorder Gene Regulates Mood-related Behaviors and Stress Reactivity in Mice

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Background: The ankyrin 3 gene (ANK3) is among the strongest and most replicated risk genes for bipolar disorder (BD) identified by patient genome-wide association studies (GWAS) and targeted association studies. ANK3 encodes the ankyrin G scaffolding protein that has many important functions in the brain, including development and maintenance of the axon initial segment (AIS) and Nodes of Ranvier of neurons that elicit and propagate action potentials, maintenance of neuronal polarity and synaptic stability, and adult neurogenesis. However, the mechanism by which ANK3 contributes to BD risk is unknown, and there are no published studies investigating the disease-relevant role of ANK3 in animal models.
Methods: To gain insight into the potential disease relevance of *ANK3*, we examined the function of mouse Ank3 in the regulation of mood-related behaviors using genetic, neurobiological, pharmacological, and gene-environment interaction (GxE) approaches. Lentiviral-mediated RNA interference was used to reduce Ank3 expression in hippocampus of adult male C57BL/6J mice. Lentivirus expressing one of two short hairpin RNA (shRNA) sequences targeting Ank3, or a scrambled control sequence, was injected bilaterally ($\geq$10E9 infection units/ml) into hippocampus dentate gyrus (N = 8-12 mice per group). Mice were assessed 14 days later for behaviors modeling bipolar disorder symptoms, including novelty- and psychostimulant-induced hyperlocomotion, motivated behavior, as well as intermediate phenotypes (prepulse inhibition), anxiety-like behaviors, circadian activity, learning (fear conditioning, habituation), and sensory and motor performance. Reversal of the behavioral phenotype was examined following chronic treatment with lithium (85 mg/kg i.p., 14 days). Expression of ankyrin G at neuronal AIS was measured by immunohistochemistry and confocal microscopy of fixed brain. Findings from the RNA interference studies were confirmed using a knockout mouse line with disruption of brain-specific Ank3 isoforms (N = 7-18 mice per group). The knockout line was also used to examine the response to chronic stress (prolonged isolation housing) and HPA axis function, which are abnormal in BD.
Results: RNA interference of Ank3 in dentate gyrus using either shRNA sequence targeting Ank3 resulted in consistent and specific alterations in anxiety tasks (elevated plus maze and light/dark transition), marked by 60-75% shorter latencies to enter aversive areas in Ank3 knockdown mice compared to control shRNA mice ($P < 0.05$). The behavioral changes are suggestive of reduced anxiety-like behavior, which may be interpreted as increased risk-taking behavior, a feature of BD mania. Other behaviors were unchanged, including locomotion, prepulse inhibition, and learning and habituation, indicating the phenotype is not due to global impairment. Chronic lithium treatment attenuated the behavioral alterations of Ank3 knockdown mice to levels on par with control mice. By immunohistochemistry, both Ank3 shRNA sequences reduced ankyrin G expression at the AIS by 40-50%, suggesting impaired action potential firing may underlie the behavioral changes, although other Ank3 functions could be perturbed. Ank3 +/− heterozygous knockout mice also exhibited significantly decreased latencies to enter aversive areas in three anxiety tasks, as well as 53% greater preferencefor sucrose over water, indicative of dysregulated motivation that is a feature of BD, compared to wild-type Ank3 + / + mice ($P < 0.05$). Chronic stress induced a switch in the behavioral phenotype, with stressed Ank3 + /− mice no longer exhibiting low anxiety and dysregulated motivation, and instead displaying increased depressive-like behavior. The transition in behavior following chronic stress has been reported in few other putative animal models of BD. Ank3 + /− mice had significantly elevated basal corticosterone levels compared to Ank3 + / + mice, and increased corticosterone levels that took longer to normalize following an acute stressor, indicating that reduced Ank3 expression is associated with altered HPA axis activity.
Conclusions: This study defines a previously unknown role for ANK3 in the regulation of mood-related behaviors and stress reactivity that lends support for its involvement in BD. This work also establishes a general framework for determining the disease relevance of genes implicated by patient genome-wide association studies.
Keywords: bipolar disorder, mouse, behavior, stress

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M175. Evidence for Diminished Interoceptive Response to Soft Touch in Adolescent and Adult Stimulant Users

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Background: Interoception, i.e. sensing of the physiological condition of the body (Craig, 2002), representation of this internal state (Craig, 2009) within the context of ongoing activities, and the initiation of motivated action to homeostatically regulate the internal state (Craig, 2005) has emerged as one of the important processes that may contribute to the susceptibility to drug abuse (Paulus *et al*, 2009). One approach to examine interoceptive sensitivity is via mechanic stimulation of the skin. Within the skin, CT (C tactile) afferents are a distinct type of unmyelinated, low-threshold mechano-receptive units, which have been found abundantly in the hairy skin but have not been found in glabrous skin of humans and other mammals (Olausson *et al*, 2002; Olausson *et al*, 2008; Vallbo *et al*, 1999). CT fibers in hairy skin may be part of a system for processing pleasant and socially relevant aspects of touch (McGlone *et al*, 2007). Attenuated interoceptive sensitivity has been proposed as a potential mechanisms of dysregulation in individuals prone to substance use (Paulus *et al*, 2009), thus this investigation aimed to examine the hypothesis that stimulant users show reduced activation in insular cortex during stimulation of CT afferents.
Methods: Two studies were conducted in (1) a group of recently abstinent methamphetamine (MD) dependent subjects and (2) a group of adolescents ages 15-17 with substance use disorders (SUD). In both studies substance users were contrasted with healthy comparison subjects (CTL). During the functional magnetic resonance imaging (fMRI) session, interoception was probed with a Soft Touch paradigm that combines a continuous performance task and with cued positively valenced interoceptive stimulation to examine the effects of anticipation and administration of interoceptive stimulation. The continuous performance task ensures the subject is attending to the visual stimuli, and is designed to keep the cognitive load relatively low but measurable using response latencies. Subjects press a button corresponding to the direction pointed by an arrow on the screen. Throughout the task, subjects are presented with one of three conditions: (1) a baseline condition, during which the individual performs the continuous performance task; (2) an anticipation condition, when the stimulus characteristics of the arrow change to signal the impending presentation of a tactile stimulus; (3) the stimulus condition, when subject receives a soft touch to the forearm or the palm. The soft touch was applied using a brush with a force of approximately 0.1 N, which was determined by the weight of the brush, along 4-cm of the palm and forearm in 2 seconds (speed of 2 cm/s).
Results: In study 1, CTL showed greater activation than MD across trials in the middle frontal gyrus, bilateral anterior insula, caudate and thalamus. In the anterior insula, greater reaction time correlated with lower activation for MD. CTL showed the opposite pattern with greater reaction time correlating with greater insular activation. MD exhibited greater caudate activation than CTL during anticipation of the upcoming soft touch but lower activation than CTL during the soft touch condition. Within MD, higher VAS pleasantness ratings were associated with (1) higher caudate activation during palm and forearm anticipation but (2) lower caudate activation during palm and forearm soft touch. In study 2, across all conditions, SUD displayed less left insula activation than CTL. During the stroke conditions, SUD exhibited more left caudate activation but less left inferior frontal gyrus activation than CTL. Whereas, for CTL, greater left inferior frontal gyrus activation was associated with higher pleasantness ratings, it was linked to higher unpleasantness ratings for teens with SUD.
Conclusions: Both study 1 and study 2 revealed that substance users show attenuated interoceptive processing and decreased reward responsivity during a pleasant interoceptive stimulus. These findings are consistent with the view that these individuals may experience a reduced sensitivity to pleasant stimuli and thus may seek stimulant drugs to enhance the experience of afferent interoceptive stimuli.
Keywords: Interoception Drug abuse Neuroimaging Amphetamine
Disclosure: M. Paulus, Nothing to Disclose; S. Tapert, Nothing to Disclose; J. Stewart, Nothing to Disclose; A. May, Nothing to Disclose; R. Migliorini, Nothing to Disclose.

M176. Sex Differences in Fear Learning and Memory: A Role for Dopamine

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Background: Women are twice as likely as men to develop Post-Traumatic Stress Disorder (PTSD), which is often characterized by a decrease in ability to extinguish fear memories. Previous research in animal models has established sex differences in the ability to extinguish conditioned fear, but the neurochemical mechanisms underlying these differences are less clear. The infralimbic (IL) region of the medial prefrontal cortex (mPFC) is potently modulated by dopamine and mediates extinction retrieval, but dopamine's contribution to extinction in female animals is not known. Here we tested the hypothesis that local dopamine D1 stimulation in the IL during extinction learning would affect extinction retrieval differently in male and female rats.
Methods: Adult male and female Sprague Dawley rats underwent stereotaxic surgery for cannula implantation targeting the IL. After recovery from surgery, animals underwent standard cued fear conditioning, extinction learning, and extinction retrieval testing. Immediately before extinction learning, animals were infused with either 0.12 μg D1 agonist SKF38393 or saline vehicle.
Results: D1 infusion did not affect extinction learning in either males or females. However, when tested the next day for extinction retrieval, females that received drug froze significantly more than those that received vehicle, while the drug had no effect in males.
Conclusions: These findings suggest that the IL in females may be distinctly sensitive to dopamine D1 stimulation. Our results hold implications for understanding sex differences in the neuromodulation of fear behavior and learning, and may inform the development of novel treatments for PTSD in women.
Keywords: sex differences, dopamine D1 receptor, extinction, infralimbic, fear
Disclosure: R. Shansky, Nothing to Disclose; C. Rey, Nothing to Disclose.

M177. Major Depressive Disoder with Mixed Features: Interim Baseline Characteristics of Subjects Enrolled in a 6 Week, Double-blind, Placebo-controlled Trial of Lurasidone

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Background: The proposed DSM-5 criteria define major depressive disorder with mixed features (MDD-MF) as one or more major depressive episodes that are associated with at least 3 of 7 manic or

hypomanic symptoms, but not meeting full criteria for mania. MDD-MF may have a more severe course of illness than other forms of MDD, and may achieve less response to standard treatments for MDD. Few studies have been conducted in patients with MDD-MF (Keck et al, Am J Psychiatry 2003;160:741-8). To further explore the characteristics of patients with MDD-MF, we present here blinded, interim baseline data on subjects enrolled in a clinical trial designed to evaluate the efficacy of lurasidone in MDD-MF.

Methods: Subjects enrolled met DSM-IV-TR criteria for MDD, had a Montgomery-Asberg Depression Rating Scale (MADRS) score ≥ 26, and met two or three of the proposed DSM-5 manic symptom criteria for a mixed state (elevated/expansive mood, inflated self-esteem/ grandiosity, flight of ideas/racing thoughts, increased/ excessive activities, pressured speech, decreased need for sleep, increased energy). Subjects were excluded if they had a lifetime history of bipolar I manic or mixed manic episode, had attempted suicide in the previous 3 months, or were deemed to be otherwise at risk for suicide. Eligible subjects completed a 3-14 day screening period prior to being randomized to 6 weeks of double-blind treatment with flexible doses of lurasidone (20-60 mg/d) or placebo. At baseline, all subjects were evaluated using the MADRS, the Clinical Global Impression–Severity of Illness scale (CGI-S), the Young Mania Rating Scale (YMRS), Hamilton Rating Scale for Anxiety (HAM-A), and the Sheehan Disability Scale (SDS).

Results: The current baseline sample consists of 48 subjects, out of a planned total of 200, with a mean age of 42.8 years. The majority of subjects were female (72.9%), Caucasian (58.3%), and reported a mean of 6.3 previous major depressive episodes (MDE), with a mean of 4.7 MDEs associated with mixed features. The mean duration of the current MDE was 4.7 months. Among first degree relatives, subjects reported that the most frequent maternal psychiatric disorders were depression (72.2%), bipolar disorder (11.1%), and anxiety disorder (11.1%); and the most frequent paternal psychiatric disorders were depression (36.8%) alcohol dependence/abuse (36.8%), and substance abuse (10.5%). Mean baseline MADRS total score was 33.3, mean CGI-S score was 4.6, mean YMRS total score was 12.9, and mean HAM-A total score was 16.8. Two current manic symptoms were reported by 62.5% of subjects, 3 manic symptoms were reported by 35.4% of subjects, and 1 subject (2.1%) reported 4 manic symptoms. The most frequent manic symptoms were flight of ideas/racing thoughts (81.2%), more talkative/pressured speech (68.8%), increased/ excessive pleasurable activities (29.2%), elevated mood (18.8%), and decreased need for sleep (16.7%). Non-specific manic symptoms, not included as core MDD-MF criteria, were also common at baseline and consisted of distractibility (75.0%), irritable mood (70.8%), and psychomotor agitation (52.1%). The mean baseline SDS total score was 18.9 ($n = 28$ subjects) reflecting a significant degree of functional impairment in MDD-MF patients.

Conclusions: Both the presentation and the psychiatric/treatment history of the patients enrolled to-date support the proposed diagnostic category of MDD with mixed features as a distinct nosological entity. MDD with mixed features is challenging to treat and randomized, placebo-controlled studies such as the current trial with lurasidone are needed to evaluate, and help establish the safety and efficacy of treatments for this population.

Keywords: Major Depression with Mixed Features, lurasidone, DSM-5, Bipolar NOS

Disclosure: T. Suppes, **Part 1:** Dr. Trisha Suppes has received grant funding or study medications from AstraZeneca, Pfizer Inc. Sunovion Pharmaceuticals, and the National Institute of Mental Health. She is on an Advisory Board and receives travel reimbursement to Ad Board meetings from Sunovion Pharmaceuticals. She recieves royalties from Jones and Bartlett (formerly Compact Clinicals), **Part 4:** Dr. Trisha Suppes has received grant funding or study medications from AstraZeneca, Pfizer Inc. and Sunovion Pharmaceuticals; J. Cucchiaro, **Part 1:** Full-time employee of Sunonvion Pharmaceuticals, **Part 2:** Full-time employee of Sunonvion Pharmaceuticals, **Part 3:** Full-time employee of Sunonvion Pharmaceuticals, **Part 4:** None; A. Pikalov, **Part 1:** Full-time employee of Sunonvion Pharmaceuticals, **Part 2:** Full-time employee of Sunonvion Pharmaceuticals, **Part 3:** Full-time employee of Sunonvion Pharmaceuticals, **Part 4:** None; P. Warner, **Part 1:** Full-time employee of Sunonvion Pharmaceuticals, **Part 2:** Full-time employee of Sunonvion Pharmaceuticals, **Part 3:** Full-time employee of Sunonvion Pharmaceuticals, **Part 4:** None; S. Targum, **Part 1:** Dr. Targum has consulted with the following companies: Acadia, Affectis, Alkermes Inc., Amgen, AstraZeneca, BioMarin, BrainCells Inc., CeNeRx, Cephalon, Cypress, CTNI MGH, EnVivo Pharmaceuticals, Euthymics, Forest Research, Functional Neuromodulation Inc, Eli Lilly and Company, Johnson & Johnson PRD, Methylation Sciences Inc., NeoSync, Neurophage, Novartis Pharmaceuticals, Novartis Bioventures, Nupathe, Prana Biotechnology Ltd., Protoxtherapeutics, Roche Labs, Sunovion, Targacept, Transcept, and Wyeth Labs, **Part 2:** Dr. Targum has consulted with the following companies: Acadia, Affectis, Alkermes Inc., Amgen, AstraZeneca, BioMarin, BrainCells Inc., CeNeRx, Cephalon, Cypress, CTNI MGH, EnVivo Pharmaceuticals, Euthymics, Forest Research, Functional Neuromodulation Inc, Eli Lilly and Company, Johnson & Johnson PRD, Methylation Sciences Inc., NeoSync, Neurophage, Novartis Pharmaceuticals, Novartis Bioventures, Nupathe, Prana Biotechnology Ltd., Protoxtherapeutics, Roche Labs, Sunovion, Targacept, Transcept, and Wyeth Labs, **Part 3:** Dr. Targum has consulted with the following companies: Acadia, Affectis, Alkermes Inc., Amgen, AstraZeneca, BioMarin, BrainCells Inc., CeNeRx, Cephalon, Cypress, CTNI MGH, EnVivo Pharmaceuticals, Euthymics, Forest Research, Functional Neuromodulation Inc, Eli Lilly and Company, Johnson & Johnson PRD, Methylation Sciences Inc., NeoSync, Neurophage, Novartis Pharmaceuticals, Novartis Bioventures, Nupathe, Prana Biotechnology Ltd., Protoxtherapeutics, Roche Labs, Sunovion, Targacept, Transcept, and Wyeth Labs, **Part 4:** Dr. Targum has consulted with the following companies: Acadia, Affectis, Alkermes Inc., Amgen, AstraZeneca, BioMarin, BrainCells Inc., CeNeRx, Cephalon, Cypress, CTNI MGH, EnVivo Pharmaceuticals, Euthymics, Forest Research, Functional Neuromodulation Inc, Eli Lilly and Company, Johnson & Johnson PRD, Methylation Sciences Inc., NeoSync, Neurophage, Novartis Pharmaceuticals, Novartis Bioventures, Nupathe, Prana Biotechnology Ltd., Protoxtherapeutics, Roche Labs, Sunovion, Targacept, Transcept, and Wyeth Labs; A. Loebel, **Part 1:** Full-time employee of Sunonvion Pharmaceuticals, **Part 2:** Full-time employee of Sunonvion Pharmaceuticals, **Part 3:** Full-time employee of Sunonvion Pharmaceuticals.

M178. The Novel Brain Penetrant NPS Receptor Antagonist, NCGC00185684, Blocks Alcohol-induced ERK-phosphorylation within the Central Amygdala, and Decreases Alcohol Self-administration in Rats

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Background: Neuropeptide S (NPS) and its receptor (NPSR) are expressed in brain areas involved in regulation of wakefulness, anxiety-related behaviors, and memory function. Thus, NPS acts in the brain as a unique transmitter: an activating anxiolytic. In addition, it has recently been found that central administration of NPS promotes cue-induced reinstatement of cocaine as well as alcohol seeking, without affecting the reinstatement of responding to water-paired stimuli. These results suggest a possible role for the NPS-NPSR system in addiction, and identify the NPSR as a

putative treatment target in addictive disorders. Understanding the role of endogenous NPS in addiction-related behaviors has, however, been hampered by a limited availability of brain penetrant NPSR antagonists. In cells expressing the recombinant NPSR receptor, NPS both stimulates intracellular calcium levels as well as cAMP accumulation, indicating that the NPSR can signal via both Gq and Gs to increase cellular excitability. In addition, NPSR activation indcues phosphorylation of extracellular regulated kinase (ERK; also called mitogen-activated protein kinase) through a direct interaction with beta-arrestin or activation of g-protein signaling pathways. Here, we examine the effects of a novel NPSR-antagonist, NCGC00185684, on alcohol related behaviors in Wistar rats. NCGC00185684, given systemically, is examined with regards to effects on alcohol and saccharin self-administration, cue- and stress-induced relapse to alcohol seeking, and sensitivity to the sedative effects of alcohol. Additionally, we examine in cell-culture the effect of NCGC00185684 on the intracellular levels of cAMP and calcium, as well as ERK phosphorylation. Further, we evaluate the efficacy of NCGC00185684 in blocking alcohol induced ERK-phosphorylation *in vivo*.

Methods: *In vitro models:* Chinese hamster ovary (CHO) cells were used for calcium and cAMP assays, as well as *in vitro* ERK phosphorylation studies. Intracellular cAMP was measured using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay with an HTRF cAMP kit (Cisbio, MA). Intracellular calcium was measured using the non-wash calcium assay Fluo8 kit (AAT Bioquest, CA), and pERK1/2 levels were measured using a TR-FRET assay by the HTRF Cellul'erk kit (Cisbio, MA). *In vivo models:* NCGC00185684 effect on ERK phosphorylation *in vivo* was measured by immunohistochemistry (IHC) in rat brain. NCGC00185684 was administered 2 hrs prior to intraperitoneal administration of alcohol (1 g/kg), animals euthanized by transcardiac perfusion, and IHC according to established protocol. For behavioral evaluation of NCGC00185684, Wistar rats were used in the following paradigms: operant alcohol self-administration and cue/stress- induced reinstatement of alcohol-seeking, progressive ratio responding, loss of righting reflex, and locomotor activity.

Results: At a dose of 1 mg/kg, NCGC00185684 significantly reduced responding for alcohol, and decreased progressive-ratio breakpoints, indicating reduced rewarding properties of alcohol. Suppression of alcohol self-administration was behaviorally specific, since the 1 mg/kg NCGC00185684 dose did not influence cue- or stress induced reinstatement of alcohol-seeking, locomotor activity, or loss of righting reflex following administration of a sedative alcohol dose. At the 1 mg/kg dose, NCGC00185684 additionally blocked alcohol-induced ERK phosphorylation within the central amygdala and nucleus accumbens shell, but not within the nucleus accumbens core or the bed nucleus of the stria terminalis. In cell culture, NCGC00185684 potently inhibited NPS stimulated intracellular calcium, cAMP, and ERK responses with IC_{50} values 36.5 ± 6.4, 22.1 ± 1.9, and 9.3 ± 11.5 nM.

Conclusions: These results suggest that antagonism at the NPSR holds promise as a treatment for aspects of alcohol use disorders. NCGC00185684 selectively blocked alcohol induced pERK in a region, the central amygdala, where ibotenic acid lesions have been shown to decrease alcohol consumption. Additionally, blockade of ERK phosphorylation was seen in the nucleus accumbens shell, a region shown to be important in operant reinstatement of drug-seeking. At the cellular level, NPS stimulates multiple signaling pathways including intracellular calcium, cAMP and ERK phosphorylation. The results presented here suggest that the NPSR antagonist NCGC00185686 preferentially inhibits, via biased antagonism, the NPS stimulated ERK phosphorylation pathway.

Keywords: neuropeptide S receptor, antagonist, alcohol dependence, ERK-phosphorylation

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M179. 1H-[13C]-Nuclear Magnetic Resonance Spectroscopy Measures of CP-101-606, Ro-256981 or Ketamine's Dose Effects on Amino Acid Neurotransmitter Metabolism

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Background: Ketamine, a non-competitive antagonist of the glutamate N-methyl-D-aspartate (NMDA) receptor, produces anesthesia with decreased metabolism at high doses but at low doses is associated with cognitive deficits, perceptual alterations, delayed antidepressant-like effects and increased metabolism. Despite widely reported clinical and preclinical studies of rapid antidepressant actions of NMDA receptor antagonists, there has been relatively little work examining the effects on neuronal oxidation or glutamate-GABA/glutamine cycling. The objective of this study was to determine how various doses of ketamine, Ro-25, 6981, and CP-101,606 alter glutamate and GABA neurotransmitter cycling in a manner consistent with its proposed antidepressant-like effects.

Methods: Male Sprague-Dawley rats ($\sim$180–220 g) were prepared with tail vein catheters under isoflurane anesthesia. For this study of three drugs at 3 different doses (8 rats/group), a total of 96 rats were acutely treated with ketamine (positive control), CP-101-606 (traxoprodil), or Ro-256981, versus vehicle (control). Animals (8 rats per group) were allowed to recover from anesthesia for at least 30 minutes before receiving intraperitoneal injections of ketamine hydrochloride (1, 30 or 80 mg/kg in vehicle; DMSO/5%, PG/10%, MC/15% of 0.25%, H2O/70%), Ro-25,6981 (3, 10 or 30 mg/kg in vehicle), CP-101,606 (traxoprodil) (1, 3 or 80 mg/kg in vehicle) or vehicle. Ten minutes after injection of ketamine, thirty minutes after injection of CP-101-606 (traxoprodil) and Ro-256981, or vehicle, a solution of [1,6-$^{13}C_2$]glucose (99 atom%; Cambridge Isotopes, Andover, MA) dissolved in water (0.75 mol/L per 200 g body weight) was infused for 8 minutes (1) through the catheter as previously described (2). resulting in rapid and constant elevations of ^{13}C labeled glucose concentrations and ^{13}C enrichments in the blood. The short period of label infusion ensured that ^{13}C incorporation into brain amino acid pools was proportional to the tricarboxylic acid (TCA) cycle rate of the respective cell (neuron and glia) types. At the appropriate time rats were quickly sedated and euthanized by focused-beam microwave irradiation, stopping all further metabolisms. The medial prefrontal cortex (mPFC) was carefully dissected and frozen in liquid nitrogen, along with heart blood drawn immediately after death. The brain tissue was extracted in methanol/HCL and ethanol, centrifuged, and the supernatants lyophilized. Samples were dissolved in phosphate buffered deuterium oxide. The concentration and ^{13}C enrichments of glutamate, GABA and glutamine were determined using ^{1}H-[^{13}C] NMR spectroscopy at 11.74 Tesla. Percentage ^{13}C enrichments of brain amino acids were normalized by their respective blood ^{13}C enrichment (3).

Results: Ketamine (30 mg/kg) produced stereotyped progressive behavioral responses, including back and forth head movements and ataxia, followed by a period of hyperactivity. Within 2 minutes of injection, ketamine (80 mg/kg) resulted in immobility, but not loss of tail pinch reflex, for the majority of animals tested, however ketamine (1 mg/kg), Ro-25, 6981 (1, 3 or 10 mg/kg) or CP-101,606 (3, 10 or 30 mg/kg) had no visually apparent behavioral effects.

Total concentrations of glutamate (Glu), GABA, and glutamine (Gln) in mPFC were unaffected by all doses of ketamine, Ro-25, 6981 or CP-101, 606 ($P > 0.2$) tested. Preliminary analysis revealed that the percentage ^{13}C enrichments of Glu-C4, GABA-C2 and Gln-C4 were higher in ketamine (30 mg/kg)-treated rats (10% to 38%, $p < 0.05$), Ro-25, 6981 (10 mg/kg)-treated rats (11% to 22%, $p < 0.05$), and CP-101, 606 (3 mg/kg)-treated rats (8% to 15%, $p < 0.05$). From the increased labeling of Gln-C4/GABA-C2 and Gln-C4 respectively it could be inferred that both neuronal TCA cycle rate and Glu/GABA-Gln cycling were increased by ketamine (30 mg/kg), Ro-25, 6981 (10 mg/kg) and CP-101,606 (3 mg/kg), suggesting the effects are truly related to the drugs actions on oxidative metabolism and amino acid cycling in the neuronal cells.
Conclusions: The studies demonstrate Ketamine (30 mg/kg), Ro-25, 6981 (10 mg/kg) and CP-101,606 (3 mg/kg) dose dependently, acutely increase Glu, Gln and GABA labeling from [1, 6-$^{13}C_2$] glucose in the rat medial prefrontal cortex, consistent with increased neuronal oxidative metabolism and increased neurotransmitter glutamate/GABA-glutamine cycling. Although there is some evidence to suggest the doses of Ro-24,6981 and ketamine that increase labeling are similar to the doses that have peak antidepressant-like effects, future studies will need to confirm the relationship. References: 1. Chowdhury et. al., Biol Psychiatry. 2012 Jun 1; 71(11):1022-5. 2. Chowdhury et. al., J Cereb Blood Flow Metab. 2008 Dec; 28(12):1892-7. 3. Chowdhury et. al., J Cereb Blood Flow Metab. 2007 Dec; 27(12):1895-907.
Keywords: GABA; glutamate/glutamine-cycle; Ro-256981; CP-101,606; ketamine; MRS; medial prefrontal cortex; NMDA
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M180. Melanocortin 4 Receptor Signaling in the Ventral Striatum Affects Obsessive Compulsive Disorder-like Behaviors in Mice

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Background: The melanocortin 4 receptor (MC4R) has previously been shown to affect depression- and anxiety-like behaviors in mice, however little is known about the role of MC4R signaling in the related syndrome Obsessive Compulsive Disorder (OCD).
Methods: Using a mouse line that expresses GFP under control of the MC4R-promoter, we first conducted a detailed neuroanatomical analysis of MC4R expression in the striatum, a region of the brain known to be important in the development of OCD symptoms. Utilizing Cre-Lox technology, MC4R expression was specifically manipulated in the striatum to directly determine the function of MC4R signaling in this brain region. These mice were then tested for behaviors thought to be relevant to the pathophysiology of OCD including learning of procedural memories and repetitive behaviors.
Results: We demonstrate that MC4R is co-expressed with the dopamine 1 receptor (D1R) in the nucleus accumbens core and shell. Furthermore, MC4R signaling in D1R neurons is required for learning both operant responding for high fat diet and a cued-version of the watermaze. Finally, loss of MC4R signaling normalizes grooming in the SAP90/PSD 95 associated protein 3 (Sapap3)-null mouse model of compulsive grooming.
Conclusions: These data identify a novel requirement for MC4R signaling in the ventral striatum for both procedural memory learning and compulsive grooming- two OCD-related behaviors. Further study of MC4R signaling in the striatum may lead to an improved understanding and treatment of OCD and OCD-related disorders such as Anorexia Nervosa and Depression.
Keywords: obsessive-compulsive disorder, obesity, melanocortin 4 receptor, ventral striatum, Sapap3
Disclosure: P. Xu, Nothing to Disclose; H. Cui, Nothing to Disclose; B. Mason, Nothing to Disclose; A. Pieper, Nothing to Disclose; M. Lutter, Nothing to Disclose.

M181. Monoamine Modulation of Separation-induced Ultrasonic Vocalizations in Preweanling F344 Rats

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Background: Impairments in social interactions are one of the earliest signs of developmental delay in children and are important diagnostic criteria in many pediatric mental disorders. Play behavior in juvenile and adolescent rats has recently been suggested as a model to study complex social interaction, because play behavior is disrupted in a number of developmental disorders and play behavior is necessary for normal social development. A major advantage of using a play behavior model is that there are known differences in playfulness between rat strains that can be used to find genetic and neurobiological substrates for this behavior. For example, Fischer 344 rats (F344) are less playful when compared to other strains of rats, including Sprague-Dawley (SD) rats from which they were initially derived. Moreover, consistent with their decreases in play behavior, F344 rats show differences in monoamine transmission when compared to SD rats. An important disadvantage to the play behavior model is that play behavior is typically assessed in rats after weaning and peaks after postnatal day 30. This time period corresponds to late childhood to early adolescence in humans and thus, play behavior cannot be used to assess deficits in social interaction when they first appear in human children. The purpose of following study was to determine if F344 rats would show evidence of decreased social communication at a developmental period analogous to early childhood. To this end we used ultrasonic vocalizations (USVs) a form of social communication in rodents that is apparent just hours after birth. Specifically, we measured separation-induced USVs in F344 and SD rats on postnatal day 15.
Methods: On PD 15, male and female rat pups were injected with either atomoxetine (norepinephrine reuptake inhibitor, 0.3, 1, or 3 mg/kg), fluoxetine (serotonin reuptake inhibitor, 3, 10, or 30 mg/kg), GBR-12909 (dopamine reuptake inhibitor, 1.5, 5, or 15 mg/kg) or vehicle 30 min before testing. The rats were placed in heated chamber (31°C, ±1°C) and USVs was measured for 20 min. Rectal body temperatures were measured immediately after being removed from the test chamber. Afterwards the rat was anesthetized with pentobarbital (40 mg/kg) and returned to the home cage. Rats were anesthetized to prevent them from emitting USVs while in the home cage.

Results: Atomoxetine increased USV emissions while both GBR-12909 and fluoxetine decreased USV production. Strain differences were apparent in the effects of all three monoaminergic drugs. The ability of atomoxetine to increase USVs was greater in F344 rats as compared to SD rats while GBR 12909 was more effective at reducing USVs in SD rats. The ability of fluoxetine to decrease USVs also differed by strain with F344 rats showing less of a decrease in USV emissions as compared to SD. The fluoxetine strain difference, however, was only seen in male rats.
Conclusions: Preweanling F344 rats did not differ from SD rats in basal USV production suggesting USV emissions, unlike play behavior, may not be suitable as a model of impaired social communication. However, the present data do indicate that USV production in preweanling F344 rats may be useful for studying disorders caused by dysregulated or altered monoamine activity.
Keywords: ontogeny, atomoxetine, fluoxetine, social communication, monoamines
Disclosure: C. Crawford, Nothing to Disclose.

M182. Deuterium Enriched L-DOPA Displays Increased Behavioral Potency and Dopamine Output in an Animal Model of Parkinson's Disease: Comparison with the Effects Produced by L-DOPA and an MAO-B Inhibitor

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Background: Our previous work has shown that introduction of deuterium in the L-DOPA molecule (D3-L-DOPA) at metabolically critical sites results in prolonged elevation of striatal dopamine levels compared to non-modified L-DOPA (Malmlöf et al. 2008; Exp Neurol 212, 538-542). Additionally, in 6-OHDA-lesioned rats, acute D3-L-DOPA administration increased motor activation as compared to L-DOPA and chronic treatment with a substantially lower dose of D3-L-DOPA produced equal antiparkinsonian effect and reduced dyskinesias (Malmlöf et al. 2010; Exp Neurol 225, 408-415). Therefore a comparative neurochemical analysis was here performed to further elucidate the mechanism underlying the behavioral effects of D3-L-DOPA
Methods: The advantageous effects of D3-L-DOPA are in all probability related to a reduced metabolism of dopamine formed from it by the enzyme monoamine oxidase (MAO). Therefore, by means of *in vivo* microdialysis in freely moving animals, the effect of D3-L-DOPA and L-DOPA on dopamine output and metabolism were here studied in 6-OHDA-lesioned rats. The combination of L-DOPA and selegiline, a clinically used MAO-B inhibitor, was used to test the contribution of concurrent MAO-inhibition. First, the behavioral effects were evaluated by monitoring drug-induced rotation, locomotion and rearing activity and subsequently, following a wash-out period, neurochemical effects were studied by the simultaneous assays of striatal L-DOPA, dopamine, noradrenaline, DOPAC, 3-MT and HVA levels.
Results: The different treatment combinations were first evaluated for motor activation; here the increased potency of D3-L-DOPA as compared to L-DOPA was further confirmed and shown to be of equal magnitude as the effect produced by the combination of selegiline and L-DOPA. The levels of dopamine and 3-MT, the extraneuronal metabolite of dopamine, were also increased following both D3-L-DOPA and selegiline/L-DOPA administration as compared to L-DOPA administration alone.
Conclusions: Taken together, the behavioral and neurochemical effects produced by D3-L-DOPA and the combination of selegiline and L-DOPA may be attributed to a decreased MAO-B activity towards released dopamine. Adjuvant treatment with selegiline in L-DOPA treated patients with Parkinson's disease potentiates the symptomatic effect of L-DOPA and allows for a significant dose reduction. The similar effects produced by D3-L-DOPA and the selegiline/L-DOPA combination in the 6-OHDA-lesioned animals hence provides further experimental support for the potential clinical advantage of monotherapy with D3-L-DOPA in the treatment of Parkinson's disease. *Acknowledgements:* This work was supported by grants from The Swedish Research Council (grant no. 4747), The Swedish Brain Foundation, Karolinska Institutet in Stockholm, Sweden. The generous supply of D3-L-DOPA from BiRDS Pharma GmbH, Germany is also acknowledged.
Keywords: Parkinson's disease, selegiline, monoamine oxidase, 3-MT, microdialysis
Disclosure: T. Svensson, **Part 3:** AstraZeneca and Lundbeck scientific advisory board meetings, **Part 4:** AstraZeneca, Organon, Schering-Plough, Merck, Johnson&Johnson; T. Malmlöf, Nothing to Disclose; K. Feltman, Nothing to Disclose; Konradsson-Geuken, Nothing to Disclose; B. Schilström, Nothing to Disclose.

M183. Impact of Sustained Administration of Asenapine on Neuronal Activity in Monoaminergic Systems in the Rat Brain

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Background: Asenapine is a psychopharmacologic agent efficacious in the treatment of schizophrenia and mania; however, its neuronal mechanism of action remains to be elucidated. *In vitro* studies showed that asenapine has high affinity (K_i2A, D_2, α_2-adrenergic and partial agonistic action at 5-HT_{1A} receptors [3]. Furthermore, local application of asenapine has been demonstrated to increase 5-HT, NE and DA levels in the prefrontal cortex and blocks 5-HT_{2A} and α_2-adrenergic receptors [2]. However, the antipsychotic and antimanic responses of asenapine are only observed following a sustained treatment in the clinic, indicating that long-term neuronal alterations underlie the therapeutic effect of asenapine. The present study will investigate the effects of short (2 days) and prolonged (21 days) administration of a clinically relevant dose of asenapine on the catecholaminergic system.
Methods: *In vivo* electrophysiological recordings were carried out in male Sprague-Dawley rats under chloral hydrate anesthesia, after 2 and 21 days of administration of vehicle (saline) or 0.1 mg/kg/day of asenapine using osmotic minipumps implanted subcutaneously. Locus coeruleus (LC) NE and ventral tegmental area (VTA) DA neurons, and hippocampus CA3 pyramidal neurons were recorded. The selective 5-HT_{2A} agonist DOI (50-150 μg/kg, i.v.) was administered to determine responsiveness of this receptor in LC following asenapine administration. The selective α_2- and α_1-adrenoceptor antagonists idazoxan (1 mg/kg) and prazosin (100 μg/kg), respectively, were administered i.v. to determine the degree of activation of postsynaptic α-adrenoceptors in the hippocampus.
Results: After 2 and 21 days of asenapine administration, the plasma level of asenapine was on average 2.4 ± 0.3 ng/ml which is in the range of that obtained with 5 mg/kg BID in humans (C_{max} = 4.2 ng/ml) [1]. In the VTA, asenapine administered for 2 or 21 days had no effect on the mean firing of DA neurons, but there was a 50% increase in percentage of spikes in burst following 2-day administration of asenapine compared to controls. This returned to normal after long-term administration. Furthermore, the number of active DA cells per tract was significantly increased following both 2-day (65%) and 21-day (90%) administration of asenapine. In LC, 2-day asenapine administration had no effect on NE firing rate whereas 21 days significantly increased this by 25%. Systemic injection of DOI 50 μg/kg decreased the firing rate of LC NE neurons in control animals by 76% and by only 7% in 2-day asenapine-administered animals, indicating a clear blockade at this receptor. Following the 21-day regimen of asenapine, iontophoretic ejection of NE were less effective to inhibit CA3 pyramidal neuronal firing when compared to controls, indicating a partial blockade of postsynaptic α_2-adrenoceptors. The tonic activation of

the α_2-adrenoceptors by endogenous NE was not enhanced following systemic injection of idazoxan followed by prazosin.
Conclusions: The present results indicate that the firing rate of VTA DA neurons was unaltered following 2 and 21-day asenapine administration, whereas number of cells per tract almost doubled following short- and long-term administration of asenapine. The firing rate of LC NE neurons increased after 21 but not 2 days of asenapine administration. The increased firing of LC NE neurons did not lead to enhanced NE transmission in hippocampus, presumably because of partial antagonism of postsynaptic α_2-adrenoceptors. Thus it is interesting to investigate the effect of long-term asenapine administration on 5-HT transmission as a marked enhancement of 5-HT neuronal firing was shown in our preliminary experiments. [1] S. Chapel, M.M. Hutmacher, G. Haig, H. Bockbrader, R. de Greef, S.H. Preskorn, and R.L. Lalonde. Exposure-response analysis in patients with schizophrenia to assess the effect of asenapine on QTc prolongation. *The Journal of Clinical Pharmacology*, 49(11):1297–1308, 2009.[2] O.I. Frånberg, M.M. Marcus, and T.H. Svensson. Involvement of 5-HT2a receptor and α2-adrenoceptor blockade in the asenapine-induced elevation of prefrontal cortical monoamine outflow. *Synapse*, 2012.[3] R. Ghanbari, M. El Mansari, M. Shahid, and P. Blier. Electrophysiological characterization of the effects of asenapine at 5-HT1A, 5-HT2A, [alpha] 2-adrenergic and D2 receptors in the rat brain. *European Neuropsychopharmacology*, 19(3):177–187, 2009.[4] M. Shahid, G.B. Walker, S.H. Zorn, and EHF Wong. Asenapine: a novel psychopharmacologic agent with a unique human receptor signature. *Journal of Psychopharmacology*, 23(1):65–73, 2009.
Keywords: asenapine; norepinephrine; dopamine; electrophysiology; antipsychotics
Disclosure: C. Oosterhof, Nothing to Disclose; M. El Mansari, Nothing to Disclose; P. Blier, **Part 1:** Consultant for Astra Zeneca, **Part 4:** Astra Zeneca, Bristol Myers Squibb, Servier, Lundbeck, Merck.

M184. Adenosine Receptor Stimulation During Extinction Training Produces Lasting Effects on Cocaine Seeking

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Background: The persistent potential for relapse in chronic cocaine users makes treatment of addiction especially difficult. Cocaine seeking during abstinence is strongly influenced by both dopamine and glutamate neurotransmission onto neurons in the nucleus accumbens. Adenosine receptors are well positioned to modulate postsynaptic dopamine signaling and presynaptic glutamate signaling that contributes to cocaine seeking. Previous studies show that stimulation of both adenosine A_1 and A_{2A} receptors reduces reinstatement of extinguished cocaine seeking. The goal of these studies was to determine the direct effects of adenosine receptor stimulation on extinction and the persistence of this treatment on subsequent reinstatement to cocaine seeking
Methods: Rats were trained to lever press for cocaine in daily self-administration sessions on a fixed-ratio 1 schedule for 3 weeks. After one week of abstinence, lever pressing was extinguished in 6 daily extinction sessions. Immediately prior to each extinction session animals were administered the A_1 receptor agonist, CPA, the A_{2A} receptor agonist, CGS 21680, or vehicle. The persistent effects of A_1 or A_{2A} receptor stimulation during extinction training were also subsequently tested on cue-, cocaine-, and D_2 agonist (quinpirole)-induced cocaine seeking. Control experiments tested the effects of dissociating the adenosine agonist treatments from the extinction sessions on subsequent reinstatement.
Results: Adenosine A_1 receptor stimulation facilitated the extinction of cocaine seeking on days 1-3, while A_{2A} receptor stimulation had no effect on extinction responding. A_1 receptor stimulation during extinction blunted the subsequent responding to both cocaine- and quinpirole-induced cocaine seeking, but had no effect on cue-induced reinstatement. On the other hand, A_{2A} receptor stimulation during extinction training had no effect on any type of reinstatement. Dissociating adenosine A_1 receptor stimulation from the extinction sessions had no effect on either extinction responding or subsequent reinstatement.
Conclusions: These findings demonstrate that adenosine A_1 receptor stimulation both facilitates the extinction of cocaine seeking and inhibits pharmacologically-induced reinstatement of cocaine seeking. Given that A_1 receptors are localized on presynaptic glutamate terminals and on D_1-containing postsynaptic medium spiny neurons in the nucleus accumbens, stimulation of A_1 receptors may normalize aberrant cocaine-induced dopamine and/or glutamate signaling within the NAc to produce lasting changes in relapse susceptibility.
Keywords: Psychostimulant, Relapse, Adenosine receptor, Dopamine receptor, Craving
Disclosure: C. O'Neill, Nothing to Disclose; B. Hobson, Nothing to Disclose; S. Levis, Nothing to Disclose; R. Bachtell, Nothing to Disclose.

M185. Pharmacologic and Genetic Manipulation of Trace Amine-Associated Receptor 1 Signaling Demonstrates its Role in Methamphetamine-stimulated Locomotor Activity in Mice

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Background: Compelling experimental evidence implicates biogenic amine transporters, in particular the dopamine and vesicular monoamine transporters, as important targets of methamphetamine (METH). However, in 2001 *in vitro* experiments showed that METH is a potent full agonist of the orphan Gas-coupled receptor now referred to as trace amine-associated receptor 1 (TAAR1). This finding revealed a previously unrecognized gap in our knowledge about METH's pharmacodynamics and gave rise to two unconventional hypotheses: METH-activated TAAR1-mediated signaling underlies the drug's activating and rewarding properties. If *in vivo* evidence were to support these hypotheses then the current model of METH's actions would have to be rethought.
Methods: EPPTB was synthesized by two different routes and the product was confirmed by NMR spectroscopy. All other drugs were obtained from Sigma-Aldrich. Wild type and *taar1*$^{-/-}$ mice (source: KOMP) were used in all behavioral pharmacology experiments. All protocols involving mice were approved by OHSU's Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Video recordings of wild type (WT) and *taar1*$^{-/-}$ mice injected intraperitoneally with METH, bupropion (BUP), cocaine (COKE) and EPPTB, a TAAR1-selective antagonist, alone or in combination were analyzed using CleverSys and GraphPad software.
Results: EPPTB (50, 75 and 100 mg/kg) pretreatment dose-dependently inhibited METH- (3 mg/kg) stimulated locomotor activity of WT mice but had no effect on *taar1*$^{-/-}$ mice. Treatment of WT mice with BUP (50 mg/kg) or COKE (15 mg/kg) had a stimulatory effect on WT mice regardless of the presence or absence of TAAR1. The activity of WT mice treated with BUP and then METH was significantly greater than the activity of similarly treated *taar1*$^{-/-}$ mice while the effect of COKE pretreatment on METH-stimulated activity was the same whether TAAR1 was present or not. WT mice administered a combination of BUP and EPPTB before receiving METH displayed an identical activity profile to *taar1*$^{-}$ mice given iBUP and METH. These results

demonstrate TAAR1 signaling plays a significant role in the manifestation of METH-stimulated locomotor activity in the mouse. *In vitro* EPPTB had no affinity for, or effect on the dopamine, norepinephrine or 5-HT transporters while in rats, *in vivo* microdialysis data were consistent with the interpretation that EPPTB has no direct effect on the dopamine transporter.
Conclusions: Our *in vivo* results are the first to demonstrate that TAAR1 is a METH receptor in mice. Furthermore, this study is the first to conclusively link a behavior in WT mice to TAAR1-mediated signaling. A possible explanation for our finding that METH-activated TAAR1-mediated signaling *facilitates* the drug's ability to stimulate locomotor activity in intact mice will be presented. The existence of a METH-activated G protein-coupled receptor means the *in vivo* actions of this drug are more complicated than previously appreciated. Current efforts are focused on exploring the mechanism(s) of action by which METH-activated TAAR1-mediated signaling influences locomotor activity and intravenous METH self-administration in mice.
Keywords: dopamine transgenic rodent EPPTB sensitization
Disclosure: D. Grandy, Nothing to Disclose; K. Tallman, Nothing to Disclose; M. Grandy, Nothing to Disclose; A. Kimbel, Nothing to Disclose; O. Anoshchenko, Nothing to Disclose; W. Grandy, Nothing to Disclose; T. Wahl, Nothing to Disclose; A. Placzek, Nothing to Disclose; T. Scanlan, Nothing to Disclose; O. Littrell, Nothing to Disclose; W. Cass, Nothing to Disclose; G. Gerhardt, Nothing to Disclose; A. Janowsky, Nothing to Disclose; G. Mark, Nothing to Disclose.

M186. Behavioral Interactions Between mGlu5 and 5-HT2A Receptors in Mice

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Background: There is evidence that metabotropic glutamate (mGlu) receptors play a role in neuropsychiatric disorders including schizophrenia, drug abuse, and depression. In recent years, reports have emerged linking serotonergic and mGlu signaling. For example, serotonergic hallucinogens, which act as 5-HT2A receptor agonists, increase glutamate release, and mGlu2/3 agonists and mGlu5 antagonists modulate the response to 5-HT2A receptor activation. Furthermore, mGlu5 receptors regulate serotonin (5-HT) release, and some of the behavioral effects of mGlu5 antagonists are blocked by 5-HT2 antagonists. These interactions between 5-HT2A and mGlu receptors may be important to our basic understanding of the neurochemical and behavioral effects of hallucinogens and potentially to the pathophysiology and treatment approaches for neuropsychiatric disorders such as depression and schizophrenia. We have recently shown that stimulation of 5-HT_{2A} receptors increases locomotor activity in mice (Halberstadt et al., *Neuropsychopharmacology* 34: 1958, 2009); as mGlu5 receptor blockade or genetic deletion produces similar effects on locomotion in mice, we have hypothesized that 5-HT_{2A} receptors are involved in the locomotor hyperactivity induced by reductions in mGlu5 signaling.
Methods: The behavioral pattern monitor (BPM) was used to measure locomotor activity in C57BL/6J mice and in mGlu5 wild-type (WT) and knockout (KO) mice on a C57 background. We tested the effects of the 5-HT2A agonists mescaline and 2,5-dimethoxy-4-methylamphetamine (DOM), and the mGlu5-negative allosteric modulator 2-methyl-6-(phenylethynyl)pyridine (MPEP). Experiments were also conducted to determine whether behavioral effects induced by reductions in mGlu5 signaling are dependent on endogenous 5-HT and are mediated by the 5-HT2A receptor.
Results: Compared with their WT counterparts, male and female mGlu5 KO mice were hyperactive ($F(1,28) = 29.11$; $p < 0.0001$). MPEP also induced hyperactivity ($F(4,43) = 8.28$; $p < 0.0001$), an effect that was absent in mGlu5 KO mice. Likewise, activation of 5-HT2A receptors with mescaline or DOM increased locomotor activity. Importantly, the locomotor hyperactivity in mGlu5 receptor KO mice was attenuated by the selective 5-HT2A antagonist M100907 ($F(1,28) = 15.12$; $p < 0.0006$). Moreover, pretreatment with M100907 completely blocked the hyperactivity induced by MPEP. We also found that MPEP did not induce hyperactivity in mice after depletion of 5-HT with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine methyl ester (PCPA). Although the locomotor hyperactivity induced by DOM was potentiated in mGlu5 receptor KO mice ($F(1,28) = 15.12$; $p < 0.0006$), MPEP did not potentiate the hyperactivity induced by mescaline.
Conclusions: These studies demonstrate that hyperactivity occurs in mice after 5-HT2A receptor activation or loss of mGlu5 receptor activity (either pharmacologically or by gene deletion). Furthermore, the hyperactivity associated with reductions in mGlu5 signaling is mediated by the 5-HT2A receptor, and is dependent on endogenous 5-HT. Because previous studies have shown that mGlu5 antagonists increase 5-HT release, the present findings indicate that reductions in mGlu5 receptor activity induce hyperactivity by increasing 5-HT release, which in turn activates 5-HT2A receptors. Although mGlu5 gene deletion potentiates the response to 5-HT2A activation, this effect does not appear to play a role in the locomotor hyperactivity because mGlu5 pharmacological blockade failed to potentiate 5-HT2A-mediated responses. Taken together, these studies demonstrate that functional interactions occur between mGlu5 and 5-HT2A receptors, confirming previous reports that the 5-HT2A receptor is responsible for mediating some of the behavioral effects associated with mGlu5 receptor blockade. Given the putative therapeutic effects associated with mGlu5 blockade (e.g., antipsychotic and antidepressant effects), further investigation is clearly warranted to characterize the functional interactions between mGlu5 and 5-HT2A receptors.
Keywords: serotonin; hallucinogen; depression; schizophrenia; locomotor
Disclosure: A. Halberstadt, Nothing to Disclose; S. Powell, Nothing to Disclose; M. Geyer, Nothing to Disclose.

M187. Chronic Exposure to Cocaine Produces Persistent Residual Effects on Functional Brain Activity in the Prefrontal Cortex: Evidence from a Nonhuman Primate Model of Cocaine Self-administration

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Background: Numerous studies have documented serious functional, neurochemical, physiological and cognitive deficits among cocaine addicts. While these deficits are often thought to be attributable to drug use, it is impossible to exclude the impact of such factors as the concomitant use of other legal and illegal drugs, poor nutrition, and, most importantly, conditions that predate drug use. It is also not clear what the contribution of environmental cues vs. the actual pharmacological effects of cocaine are on these functional changes. Much of our previous research has studied brain changes during cocaine self-administration. The goal of this study, therefore, was to evaluate the consequences of prolonged cocaine exposure on basal or "resting" brain activity, when studied in a neutral environment, in a nonhuman primate model of cocaine self-administration using the 2-[^{14}C]deoxyglucose (2DG) method.
Methods: Rhesus monkeys self-administered cocaine (0.3 mg/kg/infusion, 30 infusions per day, 100 daily sessions) under a fixed interval-3 min schedule. One or thirty days following the last drug exposure, 2DG was injected in a familiar neutral setting not previously associated with reinforcement. Rates of cerebral glucose metabolism (CMR_{glc}) in the prefrontal cortex were compared to

those of control animals, whose responding was reinforced by food pellets under identical schedules of reinforcement.
Results: Globally, CMR_{glc} throughout the prefrontal cortex was lower in cocaine-exposed animals either 1 or 30 days following their last drug exposure when compared to those of drug-naïve monkeys. These differences were most evident in ventromedial cortical areas, at both the orbital (Areas 11, 12, 13) and medial areas (Areas 32 and 24). In addition, there were significant differences between groups in the dorsolateral prefrontal cortex. These are areas in which cocaine administration itself produces significant changes in metabolism. In addition, decreased rates of CMR_{glc} were observed in Area 46 of the dorsolateral prefrontal cortex. In general these effects were more pronounced in the more rostral portions of the prefrontal cortex.
Conclusions: Thus, chronic exposure to high doses of cocaine resulted in profound and persistent residual effects on functional brain activity in the prefrontal cortex. These data suggest that differences between cocaine users and healthy controls are directly attributable to the effects of cocaine exposure, and may help to explain many of the cognitive and other behavioral deficits seen in these populations.
Keywords: cocaine prefrontal cortex imaging functional brain activity
Disclosure: L. Porrino, Nothing to Disclose; T. Beveridge, Nothing to Disclose; H. Smith, Nothing to Disclose; M. Nader, Nothing to Disclose.

M188. Uncoupling Alpha7 nACh-NMDA Receptor Complex Blocks Cue-induced Reinstatement

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Background: Smoking is the leading preventable cause of disease, disability, and premature death. Nicotine, the main psychoactive drug in tobacco, is one of the most heavily-used addictive substances. Its continued use is driven through activation of nicotinic acetylcholine receptors (nAChRs). Despite harmful consequences, it is difficult to quit smoking due to its positive effects on mood and cognition that are strong reinforcers contributing to addiction. Furthermore, a formidable challenge for the treatment of nicotine addiction is the high vulnerability to relapse following abstinence. There is no currently available smoking cessation product able to achieve greater than a 20% smoking cessation rate after 52 weeks and there are no medications that directly target the relapse process.
Methods: Nicotine self-administration and reinstatement of nicotine seeking. Locomotor activityCo-immunoprecipitation. Affinity purificationIn vitro binding assay.
Results: We report here that the α7 nACh receptor (α7 nAChR) forms a protein complex with the NMDA glutamate receptor (NMDAR) through a direct protein-protein interaction. Chronic nicotine exposure promotes α7 nAChR-NMDAR complex formation. Interestingly, administration of an interfering peptide that disrupts the α7 nAChR-NMDAR complex decreased extracellular signal regulated kinase (ERK) activity and blocked cue-induced reinstatement of nicotine-seeking in rat models of relapse, without affecting nicotine self-administration or locomotor activity.
Conclusions: Our results may provide a novel therapeutic target for the development of medications for preventing nicotine relapse.
Keywords: Nicotine relapse α7 nACh receptor NMDA receptor protein-protein interaction Interfering peptide
Disclosure: S. Li, Nothing to Disclose; Z. Li, Nothing to Disclose; A. Le, Nothing to Disclose; F. Liu, Nothing to Disclose.

M189. Activation of the μ-δ Opioid Receptor Heteromer in the Nucleus Accumbens Produces Anti-depressant-like and Anxiolytic-like Effects

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Background: Treatment-resistant major depression remains inadequately treated with currently available anti-depressants. Opioid Receptors (ORs) are involved in the pathophysiology of major depression yet remain an untapped pharmacological target. Although μ-opioid agonists such as morphine have been used in the clinic to manage refractory depression, their clinical utility is limited by the development of tolerance and adverse side effects ensuing from μOR activation. In contrast with μ- and δ-ORs, the μ-δ OR heteromeric complex has unique pharmacological and signalling properties and displays less functional desensitization, rendering it a promising therapeutic target. Although the pharmacological and molecular profile of the μ-δ heteromer *in vitro* is being elucidated, its physiological role *in vivo* is largely unknown. Recently, we demonstrated that δ-agonists bind within a novel μ-receptor ligand binding pocket and regulate μ-δ heteromer trafficking in cells. In particular, the δ-agonist UFP-512 displayed high affinity at the μ-δ heteromer. Since the δ-agonists which possess a unique pharmacology at the μ-δ heteromer also elicit anti-depressant-like and anxiolytic-like effects in animal models, we sought to investigate the role of the μ-δ receptor complex in mood regulation.
Methods: Since drugs targeting the δOR homomeric receptors may also have effects at the μ-δ heteromer, we devised a strategy to selectively analyze the effects of the μ-δ receptor complex by dissociating it. To this end, we designed a specific inhibitory peptide derived from the distal carboxyl tail of the δOR, which has been implicated in μ- and δ-OR heteromerization. To validate the use of the interfering peptide as a tool for dissociating the μ-δ heteromer, we examined its effect on μ- and δ-OR co-immunoprecitation and δ-agonist UFP-512-induced binding at, and trafficking of, the μ-δ heteromer. To determine the role of the μ-δ heteromer in mood modulation, we assessed the effect of intra-accumbens micro-injections of TAT-conjugated interfering or scrambled control peptides on the anti-depressant-like and anxiolytic-like effects of UFP-512 in the rat forced swim test (FST), novelty-induced hypophagia (NIH) and elevated plus maze (EPM) paradigms. The effects of intra-accumbens administration of the interfering and control peptides, and UFP-512 on locomotion were also assessed.
Results: *In vitro*, the interfering peptide abolished μ- and δ-OR co-immunoprecipitation and resulted in a loss of UFP-512-detected high affinity binding to, and trafficking of, the μ-δ heteromer, but had no effect on μ- or δ-OR homomers. Thus, the interfering peptide selectively dissociated the μ-δ receptor complex. Bilateral micro-injections of UFP-512 into the nucleus accumbens (NAc) resulted in reduced duration of immobility in the FST, suggestive of anti-depressant-like action. Intra-accumbens UFP-512 micro-injection also resulted in decreased latency to drink milk in the NIH paradigm and increased time spent in open arms of the EPM, suggestive of anxiolytic-like action at the level of the NAc. Bilateral micro-injections of the interfering peptide into the nucleus accumbens reversed the UFP-512-induced anti-depressant-like and anxiolytic-like actions in the FST, and NIH and EPM paradigms, respectively, whereas the control peptide had no effect. Pre-treatment with either the μ-antagonist CTOP or the δ-antagonist naltrindole abolished the anti-depressant-like effects of UFP-512 in the FST, suggesting that activation of both μ- and δ-ORs was required. Neither interfering nor inactive peptides alone affected basal responding in any of the behavioural paradigms. Imipramine and diazepam were used as positive

controls in the FST and EPM test, respectively. UFP-512 and the interfering and control peptides did not modulate locomotor behaviour.
Conclusions: Overall, our findings indicate that the μ-δ heteromer mediates the mood modulatory effects of UFP-512 in the NAc and that activation of the μ-δ heteromer in the NAc produces antidepressant-like and anxiolytic-like actions similar in magnitude to the effects of clinically relevant anti-depressants and anxiolytics in animal models of depression and anxiety. Our data also further solidify the role of the NAc in modulating depressive behaviour. By coupling to a distinct signal transduction pathway and resisting the desensitization that is characteristic of μORs, the μ-δ heteromer represents a potential therapeutic target for the management of treatment-resistant major depression.
Keywords: mu delta opioid depression anxiety
Disclosure: N. Kabli, Nothing to Disclose; T. Nguyen, Nothing to Disclose; G. Balboni, Nothing to Disclose; B. O'Dowd, Nothing to Disclose; S. George, Nothing to Disclose.

M190. Glyoxalase 1 (Glo1) Increases Anxiety in Mice by Metabolizing Methylglyoxal, which is a Novel GABAA Receptor Agonist

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Background: Numerous mouse genetic studies have identified associations between the expression of Glyoxalase 1 (*Glo1*) and anxiety-like behavior; however, the underlying mechanism has been elusive. GLO1 is an enzyme that detoxifies methylglyoxal (MG).
Methods: We created transgenic mice that overexpressed *Glo1*. We also performed pharmacological and electrophysiological studies of the effects of MG and an inhibitor of GLO1.
Results: Mice with a transgenic bacterial artificial chromosome containing *Glo1* displayed increased anxiety-like behavior and reduced MG concentrations. Acute administration of MG reduced anxiety-like behavior and, at higher doses, caused locomotor depression, ataxia, and hypothermia; effect that are characteristic of GABA-A receptor activation. When applied to primary neurons in culture, physiological concentrations of MG selectively activated GABA-A receptors with about 1/3 the potency of GABA. These effects could be blocked by the GABA-A selective antagonist SR-95531. Competition studies suggest that GABA and MG compete with one another from the same binding site.
Conclusions: Taken together our data establish that *Glo1* expression increases anxiety by reducing levels of MG, thereby altering GABA-A receptor activation. More broadly, they provide a potential link between metabolic state, neuronal inhibitory tone, and behavior. Finally, they point the way toward potentially novel pharmacological strategies.
Keywords: Anxiety, GABA-A, Glo1, CNV, Mouse, Genetics, electrophysiological
Disclosure: M. Distler, Nothing to Disclose; A. Palmer, Nothing to Disclose.

M191. A Procedure for Studying Acute, Discontinuation-induced Benzodiazepine Withdrawal in Non-human Primates

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Background: The development of physical dependence limits the therapeutic utility of benzodiazepines. In the current study, an established preclinical method for evaluating precipitated benzodiazepine withdrawal (i.e., flumazenil discrimination in diazepam-dependent monkeys) was modified in order to studydiscontinuation-induced benzodiazepine withdrawal.
Methods: Four monkeys received 5.6 mg/kg/day of diazepam and discriminated flumazenil while responding under a fixed ratio 5 schedule of food presentation. Under these conditions, flumazenil has been shown to produce discriminative stimulus and other effects that reflect the precipitation of withdrawal; however, the long (>3 days) and often variable (among individuals) duration of diazepam and its metabolites in monkeys complicates systematic study of discontinuation-induced withdrawal. On different occasions, three daily doses of lorazepam replaced diazepam for up to 9 days. On days 4 and 6 of lorazepam treatment, one or two lorazepam injections were replaced by vehicle to test whether withdrawal emerged rapidly and reliably after drug discontinuation and whether withdrawal was reversed by administration of a benzodiazepine. Plasma was collected to monitor concentrations of lorazepam, diazepam and its metabolites.
Results: Plasma concentrations of diazepam and its metabolite oxazepam decreased progressively after discontinuation of diazepam treatment and were low and near minimum levels of detection 3 days after the last injection of diazepam; another metabolite, desmethyldiazepam, was no longer evident 5 days after discontinuation of diazepam. Plasma concentrations of lorazepam increased for the first 5 days of treatment with 3.2 mg/kg/8 hr and remained stable thereafter. When lorazepam replaced diazepam, monkeys responded predominantly on the vehicle lever, indicating that substitution of lorazepam for diazepam prevented the emergence of withdrawal. Eleven hours after the last injection of 3.2 mg/kg of lorazepam (3 hr after an injection of vehicle), monkeys responded predominantly on the flumazenil lever; midazolam reversed flumazenil-lever responding in a dose-related manner. On a separate occasion, 5.6 mg/kg/8 hr of lorazepam replaced diazepam treatment; 11 hr after the last injection of lorazepam, monkeys responded only 43% on the flumazenil lever. Increasing the interval between administration of 5.6 mg/kg of lorazepam and the test session to 19 hr increased flumazenil-lever responding to 89%, and this effect was also reversed by midazolam.
Conclusions: After diazepam and its metabolites are no longer detectable in plasma (i.e., day 6), discontinuation of treatment with the relatively shorter acting lorazepam resulted in the rapid and reliable emergence of withdrawal in all monkeys, as evidenced by increased flumazenil-lever responding. The larger treatment dose of lorazepam had a longer time course, and therefore more time was needed after the last dose before monkeys responded $\geq 80\%$ on the flumazenil lever, although there was no difference in the dose of midazolam that reversed this effect. This method of substituting a shorter acting drug for diazepam provides an efficient, safe, and reliable method for systematically examining discontinuation-induced benzodiazepine withdrawal in monkeys.
Supported by USPHS grant DA09157 and DA17918 (CPF).
Keywords: benzodiazepine, dependence, withdrawal, rhesus monkey
Disclosure: L. Gerak, Nothing to Disclose; M. Javors, Nothing to Disclose; C. France, **Part 1:** Merz, Solvay, **Part 2:** Porsolt USA.

M192. Chronic Ethanol and Nicotine Co-administration, but Not Ethanol or Nicotine Self-administration, Increases the Reinforcing Properties of Nicotine within the Nucleus Accumbens Shell

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Background: The co-morbidity of alcoholism and nicotine dependency is extremely high. The use of alcohol potentiates the self-administration of nicotine, and vice versa. Recently, we have established an animal model for chronic EtOH + nicotine (Nic) co-abuse. Alcohol-preferring (P) rats will readily consumed EtOH + Nic solutions, saccharin + Nic (S + Nic) solutions, at

pharmacologically relevant levels (BECs > 105 mg%, nicotine levels > 35 ng/ml).
Methods: The current study examined the effects of chronic EtOH + Nic self-administration on the self-administration of nicotine directly into the nucleus accumbens shell (AcbSh). Adult P rats were allowed to self-administer water, EtOH, S + Nic, EtOH + Nic, or S for 10 consecutive weeks. A key component of the animal model is that EtOH and Nic intake levels are equivalent between single and co-administration groups (e.g.; EtOH intake is equivalent between EtOH and EtOH + Nic groups). Following a two week abstinence period rats were tested in standard 2-lever operant chambers (active and inactive) for the self-administration of nicotine directly into the AcbSh. Rats were randomly assigned to one of five groups (n = 6-8/group) that self-infused (FR1 schedule) modified artificial CSF (aCSF), 0.1, 0.3, 1, or 3 μM nicotine in a volume of 100 nl/infusion for sessions 1-4, aCSF for sessions 5 and 6, and the original infusate for session 7.
Results: P rats with a past drinking history of EtOH, S + Nic, S, or water all self-administered 1 or 3 μM nicotine, but not the two lower concentrations of nicotine. In contrast, only in P rats that had a past drinking history of chronic EtOH + Nic self-administration were the 0.1 and 0.3 μM nicotine concentrations self-administered directly into the AcbSh. In addition, the EtOH + Nic rats self-administered 1 and 3 μM nicotine (110 ± 8; 119 ± 11 infusions/session, respectively) at a higher rate than all other groups (highest self-administration rate in all other groups - 28 ± 4; 32 ± 5 infusions/session, respectively). Self-administration for aCSF was comparable between all five groups (range 5 ± 2-8 ± 3 infusions/session).
Conclusions: The data suggest that chronic EtOH + Nic has produced unique neuroadaptations in the AcbSh, that are not observed following equivalent self-administration of EtOH or Nic, which have greatly increased the reinforcing properties of Nic within this brain region. The co-morbidity of EtOH and nicotine co-abuse could, in part, be based upon the alterations in the AcbSh produced by EtOH + Nic co-abuse.
Keywords: ethanol nicotine co-abuse reward
Disclosure: S. Hauser, Nothing to Disclose; G. Deehan, Nothing to Disclose; J. Toalston, **Part 1:** none, **Part 2:** Indiana University: School of Medicine; J. Wilden, **Part 2:** Indiana University: School of Medicine; W. Truitt, **Part 2:** Indiana University: School of Medicine; W. McBride, **Part 1:** None, **Part 2:** Indiana University: School of Medicine; Z. Rodd, **Part 2:** Indiana University: School of Medicine, Rental Property Income.

M193. mTOR Altered by Chronic Alcohol Consumption: Effects on Signaling and Complex Formation in Mice

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Background: Excessive alcohol (EtOH)consumption is often associated with abuse and dependence. Several brain neurochemical signaling systems play a significant role in both. Moderate EtOH intake, however, is associated with reduced morbidity and mortality. There is a growing body of evidence that lifespan is extended by the suppression of mammalian target of rapamycin (mTOR). EtOH, an inhibitor of phospholipase D (PLD), prevents mTOR signaling by decreasing levels of phosphatidic acid (PA).
Methods: C57Bl/6J mice consumed EtOH by oral intake of the Lieber-Dicarli diet (LDD)(6.7 % v/v). Control animals were maintained non-EtOH LLD. Intake was maintained for at least 3 weeks. The effects of EtOH on the activity of mTORC1 were investigated by examining the activation of its downstream target, ribosomal S6 protein kinase 1(S6K1). We also examined the effect of alcohol on mTOR complex formation by immunoprecipitating mTOR, raptor and rictor. The effects were examined on several brain sites that mediate EtOH effects including alcohol intake, reinforcement, and memory.
Results: Phosphorylation levels of S6K1 were decreased in lysated prepared from cortex, hippocampus, nucleus accumbens and hypothalamus in EtOH consuming mice. Lysates prepared from EtOH consuming mouse brains showed reduced association between mTOR and raptor as well as mTOR and rictor.
Conclusions: These data suggest that the effects of chronic alcohol intake reduce mTOR signaling in the brain and that these effects may play a significant role in alcohol's role in longevity as well as its effects on behavior.
Keywords: mTOR Alcohol Intake Longevity Signalling Brain
Disclosure: M. Lewis, Nothing to Disclose; D. Salloum, Nothing to Disclose; D. Foster, Nothing to Disclose; R. Clugson, Nothing to Disclose; W. Blaner, Nothing to Disclose.

M194. Chronic Unpredictable Stress Modifies Cortical GABA and Induces Depression Like Behavior in Rats: Reversal by Ketamine

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Background: In rodents, repeated exposure to mild, unpredictable stress (CUS) induces specific behavioral modifications thought to model certain behavioral phenotypes of major depression in humans. Recent clinical observations using ^{1}H-MRS have established that GABA levels are altered (decreased) in the cortex of patients with major depressive disorder (MDD), suggesting a potential role for disrupted inhibition in the etiology and/or treatment of mood disorders. Moreover, clinical studies show prompt remission from MDD, lasting 1-2 weeks, after administration of a single non-anesthetic dose of the glutamate NMDA receptor antagonist ketamine. Despite the limited duration of a single dose of ketamine, it is a prototype for a new generation of antidepressant in that it does not directly target monoamine systems and it has a rapid onset of action. Therefore, using CUS as a potential animal model for stress-induced mood disorders, we hypothesized that CUS would alter performance in the forced swim test as well as levels of prefrontal GABA and that ketamine would reverse these effects.
Methods: After exposure to a random (unpredictable) pattern of mild stressors (swim, cage rotation, overnight isolation, lights on/off, no food/water overnight, cold isolation, restraint) twice daily for 10 days or operator handling (controls), male SD rats were treated with ketamine (40 mg/kg IP) and behavior assessed in the forced swim test (FST); 21 hours later animals were sacrificed in order to collect trunk blood as well as tissue punches from the anterior (rostral) cingulate cortex (ACC). Levels of GABA, as well as other relevant neurochemicals, were measured simultaneously *ex vivo* in intact tissue punches (~2-3 mg) using high resolution magic angle spinning ^{1}H-MRS at 11.7 T with a Bruker Avance spectrometer. Spectra were analyzed with a custom LCModel and absolute values of neurochemicals normalized to tissue weight; FST was scored by 2 independent raters. Plasma corticosterone was determined by ELISA in the CUS and control groups.
Results: Exposure to repeated stressors induced a depression-like phenotype as indicated by significantly decreased weight gain, increased plasma corticosterone, and increased immobility in the FST. When compared to controls, GABA levels increased (20-25%) in the ACC of the stressed animals. Administration of ketamine on the last day of treatment blunted the depression-like behavior and normalized GABA levels in the ACC following CUS. Ketamine did not significantly affect immobility in non-CUS (control) animals.
Conclusions: Disrupted weight gain, increased plasma glucocorticoid, and increased immobility in the FST suggest that exposure to the experimental stressors produced a physiological phenotype similar to that seen in clinical depression. Notably, the

stress-induced increase in ACC GABA is consistent with numerous reports indicating a critical role for cortical GABA in MDD. However, clinical ^{1}H-MRS studies report decreased occipital GABA in untreated MDD patients, an effect that is reversed with chronic SSRI or ECT therapy (Sanacora *et al*). Obvious dissimilarities in species, disease endophenotypes, duration of stress, brain region, and analytical technique (*in vivo v ex vivo*) may account for differences between studies. The ability of a single dose of ketamine to reverse CUS-induced phenotypes (the ACC GABA increase as well as the immobility time in the FST) is consistent with the beneficial effect of ketamine in patients with treatment-resistant MDD. Although the acute antidepressant effect of ketamine in rats relies on activation of specific pathways (AMPA receptor trafficking, BDNF, mTOR, eukaryotic elongation factor 2, and synaptic remodeling), the known effects on GABA systems is evolving. Since stress activates both glia and microglia in the cortex, disrupted glutamate / GABA-glutamine cycling would similarly disrupt GABAergic and glutamatergic signaling. Although speculative, the antidepressant-like effects of NMDA receptor blockade may be associated with normalized amino acid cycling between cortical neurons and astrocytes. **Grant/Other Support:** R01 DA016736 (MPG), K01 DA016736 (SAP), Anesthesiology Fund for Med Research, Psychiatry Joe Young Res Fund
Keywords: ketamine GABA Stress 1H-MRS forced swim
Disclosure: M. Galloway, **Part 1:** Mrs Bijal Galloway is Therapeutic Sales Manager at GlaxoSmithKline, Greater Detroit Area, **Part 2:** Mrs Bijal Galloway is Therapeutic Sales Manager at GlaxoSmithKline, Greater Detroit Area; F. Ghoddoussi, Nothing to Disclose; G. McKelvey, Nothing to Disclose; S. Perrine, Nothing to Disclose.

M195. Dendritic Spine Plasticity Induced by the mGluR5 Positive Allosteric Modulator CDPPB

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Background: Glutamate is the primary excitatory neurotransmitter in the brain and mediates numerous forms of neural plasticity including long-term potentiation (LTP) and long-term depression (LTD). Both ionotropic (iGluR) and metabotropic (mGluR) receptors are widely known to regulate synaptic plasticity. Group I mGluRs (mGluR1 and mGluR5) are positively coupled to N-methyl-D-aspartate (NMDA) receptor function, and *in vitro* and *in vivo* studies have shown that Group I mGluR positive allosteric modulators (PAMs) enhance synaptic plasticity as well as various forms of learning and memory, and reverse deficits in cognition induced by NMDA receptor antagonists. The goal of the present study was to determine if positive allosteric modulation of mGluR5 receptors produces evidence of structural plasticity at the level of dendritic spine density and morphology as assessed by diolistic neuronal labeling. The prefrontal cortex and hippocampus were chosen as regions of interest due to their involvement in behaviors modulated by mGluR5 PAMs including extinction learning and spatial memory.
Methods: Male Sprague-Dawley rats (250-275 g upon arrival) were pair housed and treated with the mGluR5 PAM 3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB, 30 mg/kg i.p., n = 5) or vehicle (10% Tween 80 in sterile saline, n = 5) once daily for 10 consecutive days. Following the last injection, rats were perfused with phosphate-buffered saline (PBS) followed by 1.5% paraformaldehyde (PFA). Brains were then removed and fixed in 1.5% PFA for an additional 30 min. Next, brains were mounted on a vibratome and coronal sections were cut at 150 μm thickness. Tissue sections were then injected diolistically with DiI-coated tungsten particles (1.3 μm diameter) using a Bio-Rad Helios Gene Gun with helium as the carrier gas. Following tissue labeling, brain sections were incubated in PBS for 24 hr at 4°C to allow for the spread of lipophilic DiI throughout the plasma membrane. Brain sections were then mounted onto microscope slides and sealed with an anti-fade mounting medium. Labeled neurons in the brain regions of interest were identified under epifluorescence microscopy, and serial z-sections of labeled dendrites were acquired at 0.1 μm step sizes on a Leica confocal laser scanning microscope. Images were then deconvolved using AutoQuant X2.2, and dendritic spine density, volume, length, and head and neck diameter were analyzed using the Filament Tracer module of Bitplane Imaris v7.4.2. Dendritic segments analyzed were at least 10 μm from the cell soma and beyond the first branch point, devoid of crossing filaments, at between 50 and 80 μm in length.
Results: Preliminary analyses of neurons labeled in the CA3 region of the hippocampus revealed that there were no differences in spine volume, length or head/neck diameter between CDPPB and vehicle treated animals. However, CDPPB-treated animals demonstrated a ~250% increase in spine density in this region. Analyses of structural changes in dendritic spines from neurons in the prefrontal cortex are currently ongoing.
Conclusions: Our findings indicate that repeated administration of an mGluR5 PAM for 10 consecutive days increased dendritic spine density in the CA3 region of the hippocampus as compared with vehicle treated animals. Although the current results are still preliminary in nature, no apparent changes in spine volume, head/neck diameter, or length were noted. The increases in dendritic spine density in the hippocampus of CDPPB-treated animals are suggestive of enhanced neural plasticity in this region, which is in agreement with our previous findings that mGluR5 PAMs enhance LTP and LTD in the hippocampus and improve performance in a spatial memory task (Ayala et al., Neuropsychopharmacology, 2009). Completion of analysis of dendritic spine plasticity in the prefrontal cortex will shed light on a possible morphological correlate of enhanced extinction learning following cocaine self-administration produced by CDPPB that we have previously observed (Cleva et al., Behav Neurosci, 2011). In conclusion, repeated administration mGluR5 PAMs produces evidence of structural plasticity that parallels known effects of these compounds on synaptic plasticity and various forms of learning and memory. These findings provide further evidence of potential pre-cognitive effects of mGluR5 PAMs that may be of potential clinical benefit for CNS disorders such as schizophrenia and drug addiction. This work was supported by NIH grant DA024355. The authors have no conflicts of interest to declare.
Keywords: dendritic spine, plasticity, morphology, mGluR5, positive allosteric modulator
Disclosure: A. LaCrosse, Nothing to Disclose; S. Taylor, Nothing to Disclose; M. Olive, Nothing to Disclose.

M196. Expression Profiles of Mitochondrial Genes in Frontal Cortex and Caudate Nucleus of Developing Humans and Mice Selectively Bred for High and Low Fear

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Background: Although genes associated with the mitochondria have been implicated in developmental and psychiatric disorders, expression profiles of those genes in human brain development and fear-related disorders remain unclear.
Methods: Using microarray data available from the public domain, we investigated the developmental expression pattern of the genes associated with mitochondrial function in the prefrontal cortex (PFC) and the caudate nucleus (CN) of psychiatrically normal subjects ranging in age from birth to 50 years.
Results: Based on the Gene Ontology analysis, many genes associated with the mitochondrion (115 genes in the PFC and 117 genes in the CN) showed expression changes across age (FDR

qv < 0.05). Interestingly, a majority of the genes in the PFC (91%), but not the CN (62%), showed a gradual increase in expression during development. Using quantitative PCR, we validated the expression changes of four genes including monoamine oxidase B (*MAOB*), NADH dehydrogenase flavoprotein (*NDUFV1*), mitochondrial uncoupling protein 5 (*SLC25A14*) and tubulin beta-3 chain (*TUBB3*). In mice, overall developmental expression pattern of these genes were similar to the pattern observed in humans ($p < 0.05$). However, mice selectively bred for high fear did not exhibit normal developmental changes of *MAOB* and *TUBB3* in the PFC.

Conclusions: These results suggest that the genes associated with the mitochondria in the PFC may play a significant role in brain development and the regulation of fear-related behavior. We demonstrate the utility of integrating data from postmortem brain tissue and an animal model to better understand the role of mitochondrial genes in fear and anxiety disorders.

Keywords: mitochondria; gene expression microarrays; oxidative phosphorylation; fear; PTSD

Disclosure: K. Choi, Nothing to Disclose; T. Le, Nothing to Disclose; J. McGuire, Nothing to Disclose; J. Coyner, Nothing to Disclose; B. Higgs, Nothing to Disclose; S. Diglisic, Nothing to Disclose; L. Johnson, Nothing to Disclose; D. Benedek, Nothing to Disclose; R. Ursano, Nothing to Disclose.

M197. Development of Antipsychotic Medications with Novel Mechanisms of Action Based on Computational Modeling of Hippocampal Neuropathology

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Background: A large number of currently used antipsychotic medications are only partially effective, and many carry debilitating side effects; additionally, there are relatively few new agents in the development pipeline. Here, we use a computational modeling approach to identify potential antipsychotics with novel mechanisms of action, using synchronization deficiencies in the gamma frequency band as a quantitative biomarker of the disease. There is evidence that gamma activity subserves particular cognitive functions, such as perceptual binding within a particular sensory modality, or integration of information from different sensory modalities, to form a coherent percept. Thus, disturbed function may be etiologically related to some of the positive symptoms of schizophrenia, such as hallucinations or compromised reality testing. In contrast to past computational modeling efforts, many of which have emphasized abstracting away biological detail, we have taken a tissue level approach, basing our work on an extensive review of the biological literature. This methodology is made possible by the availability of computing platforms with processing capacity several orders of magnitude greater than those of a generation ago (and a concomitant decrease in cost).

Methods: Using a 72-processor supercomputer, we have created a detailed hippocampal simulation, featuring multicompartmental neuron models with multiple ion channel subtypes and synaptic channels with realistic temporal dynamics. To quantify the "schizophrenic-ness" of a given model run, we instantiated a simulated steady state evoked potential (SSEP) task. Briefly, the network was driven at 20, 30, and 40 Hz, and a simulated EEG was calculated; this was analyzed via fast Fourier transform (FFT) to determine which frequencies were present, and their relative power. The degree to which this matched the pattern seen in clinical studies (i.e., the degree to which there was a specific deficit in 40 Hz response) was quantified using an illness metric. Previous work we conducted indicated that co-occuring modest reductions in NMDA system function (−30%) and dendritic spine density (−30%) produced robustly schizophrenic model behavior. To this schizophrenic model we applied the effects of 1,500 virtual medications, by implementing five medication effects in a graded and combinatorial manner. These effects were (a) manipulation of AMPA channel maximum conductance (g_{max}); (b) increase in α_2 channel function (analogous to the activity of agent MK-0777); (c) enhancement of NMDA synapse function (analogous to d-serine and related compounds); (d) alteration of the decay time constant (τ_2) of the AMPA channel; (e) increase in strength of calretinin positive (CR+) interneuron projection strength. Clearly, these fall into two categories–those that can be effected with currently know medications (a-c), and those that are more speculative (d,e). For each trial, the model was driven at 20, 30, and 40 Hz, then scored based on the degree to which it had been returned to baseline behavior, ranging from 1.0 (exactly replicating control model behavior) to 0. We also applied to the model a number of existing agents that are known not to have antipsychotic efficacy, to serve as "negative controls".

Results: In total, 97 virtual medications, or 6.5% of the 1,500 tried, produced non-zero scores. 24 received scores of 0.90 or higher. When analyzed at a single effect level, many of these most effective simulated medications *decreased* AMPA τ_2 or modestly increased NMDA activity. To understand interactions between effects, we ran a three-way analysis of variance on the 97 virtual medications. Notably, this analysis showed a very weak single factor effect for increasing CR+ projection strength, but an extremely strong interactive effect with decreases in AMPA τ_2 (the most robust interaction of all combinations tested). There was also a strong interaction between AMPA τ_2 decrease, AMPA g_{max} increase, and NMDA increase. Additionally, in negative control trials, the model accurately distinguished agents that are known to lack clinical efficacy.

Conclusions: Because schizophrenia is likely the result of system level failure, it may be possible to achieve re-equilibration by introducing changes in ways that are not simple reversals of the causative lesions. We have identified such mechanisms, which could potentially serve as targets for pharmacologic agents. Significantly, we have shown particular combinations of effects that can powerfully interact to correct aberrant behavior, something that is very difficult to predict *a priori* without this modeling approach. This suggests that it is possible that a particular experimental pharmacologic mechanism that has not appeared successful clinically (e.g., increasing AMPA g_{max} via ampakines) is not necessarily incorrect, strictly speaking, but rather incomplete—that is, in combination with other cellular level effects, amelioration of symptoms may be achieved. While this work centers on the hippocampal neuropathology underlying schizophrenia, importantly the process can be applied to other conditions for which there are modelable neurophysiologic or neurocognitive biomarkers.

Keywords: computational modeling hippocampus gamma oscillations electrophysiology drug discovery

Disclosure: P. Siekmeier, Nothing to Disclose; D. VanMaanen, Nothing to Disclose.

M198. Posttraining Optogenetic Stimulation and Inhibition of Basolateral Amygdala Activity, Respectively, Enhances and Impairs Retention of Inhibitory Avoidance Learning

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Background: The basolateral amygdala (BLA) has long been implicated in the memory consolidation for various types of learning. However, previous techniques used to manipulate BLA activity during the posttraining period have been restricted in their precision for stimulating or inhibiting neuronal activity. This has been a significant problem in understanding the processes underlying memory consolidation, as, for example, recording studies have indicated that specific firing frequencies of BLA

neurons appear to be related to learning. To gain a better understanding of how the BLA influences memory consolidation, we employed optogenetic manipulations of the BLA in order to gain more precise control of neuronal activity to influence consolidation processes for inhibitory avoidance (IA) learning.
Methods: For the stimulation experiments, the BLA of male Sprague-Dawley rats was bilaterally injected with adeno-associated virus (AAV) containing the construct for the light-sensitive cation channel channelrhodopsin (AAV5-CaMKII-hChR2(E123A)-eYFP) or the control vector (AAV5-CaMKII-eYFP). In a separate set of experiments, the BLA of rats was bilaterally injected with AAV containing the construct for the light-sensitive outward proton pump ArchT (AAV5-CAG-ArchT-GFP) or the control vector (AAV5-CAG-GFP). After two to three weeks, rats underwent a second surgery in which guide cannulae were implanted, bilaterally aimed at the BLA. One week later, rats underwent IA training, using a standard two-chamber alley. During training, rats were placed into the lit compartment and were permitted to cross into the dark compartment. When they entered the dark compartment, a door was inserted to prevent return to the lit compartment and a single footshock was administered. Immediately after training, fiber optic probes were inserted into the cannulae, with the fiber optic tips terminating ~0.5 mm prior to the center of intended illumination. Rats then received optical illumination of the BLA, using the appropriate wavelength of light (473 nm or 561 nm), to stimulate or inhibit neuronal activity. Different patterns of stimulation were used to determine the optimal frequencies of stimulation for enhancing retention. Rats' retention of their learning was assessed two days later by placing them back into the lit compartment and measuring their latencies to enter the dark compartment.
Results: Posttraining stimulation of BLA glutamatergic neurons (2 s train of 40 Hz light pulses, given every 10 s for 15 min) enhanced retention, as compared to no-illumination control rats. Rats receiving trains of 20 Hz stimulation were not significantly different from control rats or those receiving trains of 40 Hz stimulation, indicating an intermediate effect. Control experiments with identical patterns of illumination but in eYFP-alone control rats showed no effect. In addition, rats receiving optical stimulation but no footshock during training showed no difference from control rats. Posttraining bilateral inhibition of the BLA for 15 min, using ArchT, impaired retention compared to non-illuminated control rats. Inhibition for only 1 minute showed no impairment, suggesting that inhibition of BLA activity for 15 min was necessary to impair memory. In addition, inhibition of BLA neurons given 3 h after training had no effect. Control experiments with 15 min of illumination in GFP-control rats also produced no effect.
Conclusions: These findings indicate that optogenetic manipulations of neuronal activity in the BLA modulate memory consolidation, an important step in the use of optogenetics to investigate the neural circuits underlying consolidation. Critically, control experiments indicate that illumination alone had no effect on retention of training in either set of experiments. Moreover, inhibiting BLA activity 3 h after training had no effect, suggesting that the 15 min of ArchT activation did not harm the cells or produce any long-lasting effects on neuronal activity. Previous recording studies indicate that the coupling of BLA activity with downstream structures in the 40 Hz range increases across learning trials, suggesting that activity in the gamma-frequency range may be important for learning. Consistent with those findings, the present data show that stimulating BLA glutamatergic neurons using trains of 40 Hz light pulses enhances retention, providing critical evidence for the importance of activity in this frequency range during the posttraining period for strengthening memory consolidation.
Keywords: channelrhodopsin, consolidation, ArchT, memory, modulation

Disclosure: M. Huff, Nothing to Disclose; R. Miller, Nothing to Disclose; D. Moorman, Nothing to Disclose; R. LaLumiere, Nothing to Disclose.

M199. NMDA-Receptor Antagonist Ketamine Induces Brain Hyperconnectivity at Rest

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Background: Under the influence of *N*-methyl-D-aspartate glutamate receptor (NMDA-R) antagonists, healthy subjects experience schizophrenia-like positive and negative symptoms. In pre-clinical models, NMDA-R antagonists modify the action of gamma-aminobutyric acid (GABA) neurons and alter brain oscillations. In this investigation, we sought to test the hypothesis that the NMDA-R antagonist, ketamine, would modify brain oscillations as measured by functional magnetic resonance imaging (fMRI) and that these changes would be related to schizophrenia-like symptoms.
Methods: A primary sample of healthy subjects (n = 22) participated in an fMRI session during which they received ketamine. The morning of the experimental session, symptoms were rated with the PANSS. During the first scan, subjects focused on a crosshair and received a saline bolus followed by a constant saline infusion. After eight scans of a spatial working memory (WM) task (reported elsewhere), they received another bolus run but this time with a ketamine bolus of 0.23 mg/kg over 1 minute, followed by a constant infusion of 0.58 mg/kg/hr. After another eight WM runs i.e. approximately 45 minutes ketamine infusion, a partially, overlapping sample of twelve subjects (secondary sample) received an additional full resting fMRI scan with continued ketamine infusion. After completing the protocol, all subjects were removed from the scanner and their symptoms were rated again, considering the entire ketamine experience. Global brain connectivity (GBC), the average correlational strength of each voxel with all other brain voxels, was assessed during non-task runs. For the primary sample, to assure relative stability in blood plasma ketamine levels, the last 50 images of the saline and ketamine boluses were used. For the secondary sample, the full saline bolus run was compared to the full ketamine resting run.
Results: Enhanced functional connectivity was observed across the entire brain almost immediately after infusion began and after 45 minutes of continuous ketamine infusion. Negative symptoms were related to increased GBC in specific areas of the thalamus and striatum that were related to the frontal cortex. In contrast, positive symptoms were related to increased GBC in a wide range of areas, many of them involved in interoceptive or exteroceptive processing.
Conclusions: This investigation is consistent with the hypothesis that NMDA-R antagonism is associated with disruption of brain oscillations and that these alterations are related to schizophrenia-like symptoms. Thus, it is consistent with the speculation of preclinical researchers (1, 2) and gives credence to a recent theory linking disturbances in gamma to psychomimetic effects (3). This research suggests that measures of functional connectivity may provide an important biomarker in medication development and that regionally-specific information, such as can be obtained with fMRI, may be crucial in understanding the link between oscillation changes and schizophrenia-like symptoms. 1. Pinault D (2008): N-Methyl d-Aspartate receptor antagonists ketamine and MK-801 induce wake-related aberrant [gamma] oscillations in the rat neocortex. Biological Psychiatry. 63:730-735. 2. Hakami T, Jones N, Tolmacheva E, Gaudias J, Chaumont J, Salzberg M, et al. (2009): NMDA receptor hypofunction leads to generalized and persistent

aberrant gamma oscillations independent of hyperlocomotion and the state of consciousness. PLoS ONE. 4:e6755-e6755. 3. Wood J, Kim Y, Moghaddam B (2012): Disruption of prefrontal cortex large scale neuronal activity by different classes of psychotomimetic drugs. The Journal of Neuroscience. 32:3022-3031.

Keywords: NMDA-receptor antagonism, ketamine, schizophrenia, functional connectivity magnetic resonance imaging

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M200. Amygdala Projections to Lateral Bed Nucleus Stria Terminalis in Primate

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Background: The bed nucleus of the stria terminalis (BST) is a basal forebrain structure associated with producing sustained fear responses to diffuse aversive stimuli. In humans, BST activity is associated with threat monitoring, a process that is exaggerated in individuals suffering from anxiety disorders and post-traumatic stress disorders. The BST is a heterogenous structure divided into medial and lateral divisions. The lateral division (BSTL) is most associated with anxiety responses. In primates, two main components of the lateral BST are the dorsolateral division (BSTLD) and the juxtacapsular division (BSTLJ). The amygdala is a key afferent of the BST, and is also a complex structure composed of multiple subnuclei. The central nucleus of the amygdala shares a unique relation with the BST, forming a structure known as the 'central extended amygdala'. The central extended amygdala is composed of the BSTL, the central nucleus, and a column of cells that bridge the two structures. Although functional studies emphasize connectivity between the central nucleus and the BSTL, other amygdala subnuclei provide information that may differentially modulate BSTL function. One such region is the cortico-transition area (CTA), a transitional region in the ventromedial amygdala that receives hippocampal and cortical inputs which may provide input on contextual memories and interoceptive state, respectively. A significant projection from the CTA to the BSTL would suggest that emotionally relevant contextual information from the past and inner body sensations are important in directing BST activity and ultimately regulating sustained fear responses. We were also interested in determining whether the amygdala sends differential projections to BSTL subdivisions since divisions of the BSTL are thought to have different functions. While the BSTLD modulates neuroendocrine and autonomic responses to stress, the BSTLJ participates in influencing autonomic and motor circuits in order to produce adaptive responses. The two main goals of this study were to 1) identify the amygdala subdivisions that project to the BST 2) to identify whether the BSTLD and the BSTLJ have distinct amygdala projection profiles.

Methods: Nine injections of retrograde tracers into the BST in Old World Monkeys were analyzed for relative placement in the dorsolateral (BSTLD) and juxtacapsular subdivisions (BSTLJ). Localization in specific subregions was determined using subdivision-specific neurochemical markers. Retrogradely-labeled cells in the amygdala were visualized using immunocytochemistry with DAB processing, and anterograde injections in the amygdala were used for confirmation. Distribution of cells was charted using Neurolucida under brightfield microscopy with a 10x objective.

Results: Injections in both the BSTLD or BSTLJ resulted in strong retrograde labeling in the accessory basal (magnocellular subdivision), basal nucleus, and the CTA. Overall, BSTLD injections resulted in relatively more cells in these subdivisions than the BSTLJ injections. Injections including the BSTLD had additional retrogradely labeled cells in the corticomedial nuclei (medial nucleus, anterior and posterior cortical nuclei), accessory basal nucleus (parvicellular subdivision) and both divisions of the central nucleus.

Conclusions: The results of this study indicate that main sources of inputs to both components of the BSTL include the CTA and the magnocellular accessory basal nucleus (ABmc). These nuclei have important reciprocal relationships with the hippocampus: the ABmc is a key input to the hippocampus, while the CTA is a key recipient of hippocampal inputs in the primate. Projections from these amygdala nuclei to both divisions of the BSTL suggests that normal BSTL function may depend, in part, on recognizing the emotional salience and internal sensations associated with a specific context, which may ultimately be relevant in guiding an organism's state of vigilance. Our results also show that only the BSTLD receives substantial inputs from the central nucleus, which are embedded with general and substantial inputs from the CTA and accessory basal nucleus. The central nucleus is best known as an effector of autonomic and motor responses to conditioned fear. However, it is also important in orienting responses to changes in salience of a stimulus. This afferent information may be particularly important in 'monitoring' the environment for potential threat signal, which has been associated with BSTL function.

Keywords: extended amygdala, central nucleus, basal nucleus, corticoamygdaloid transition region, anxiety

Disclosure: J. Fudge, Nothing to Disclose; D. deCampo, Nothing to Disclose.

M201. Role of the Nucleus Accumbens Shell to Lateral Hypothalamic (AcbSh-LH) Pathway in "Depressive-like" Behavior in Rats.
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Background: The nucleus accumbens shell (AcbSh) and lateral hypothalamus (LH) are key brain regions involved in regulating emotion and mood-related behavior. Evidence suggests that elevated activity of AcbSh outputs is associated with aversion or negative mood. There is also evidence that pharmacological activation of the AcbSh can inhibit neurons in the LH, and that global inhibition of LH neurons increases "depressive-like" behavior on the forced swim test (FST). However, it is unknown whether the negative mood-related effects of global AcbSh activation are due to AcbSh GABAergic neurons that project directly to the LH. In order to examine this possibility, we used optogenetics to either globally activate all AcbSh outputs or to selectively activate AcbSh-LH outputs alone in order to determine their effects on "depressive-like" behavior in the FST.
Methods: An adeno-associated viral (AAV) vector was used to express either an enhanced yellow fluorescent protein (EYFP, control) or the blue-light (473 nm) activated ion channel channelrhodopsin (ChR2) protein fused to EYFP (AAV-ChR2-EYFP). In experiment 1, rats were given bilateral injections of either the AAV-ChR2-EYFP or AAV-EYFP virus in the AcbSh, and fiber optic cannula were implanted $\sim$0.5 mm above the AcbSh to allow for global stimulation of all AcbSh outputs. In experiment 2, rats were injected with either AAV-ChR2-EYFP or AAV-EYFP in the AcbSh, and fiber optic cannula were implanted $\sim$0.5 mm above the LH to allow for selective stimulation of AcbSh nerve terminals in the LH. Rats were given 3 weeks to recover to allow for sufficient AAV virus expression, after which they were subjected to a two day forced swim test procedure. On the first day, rats were placed in plastic cylinders filled with water (35 cm depth, 28-30°C) for 15 minutes. On the second day, rats received optogenetic stimulation for 30 minutes (20 Hz, 10 ms pulse duration, 200 pulses/min) and were subsequently placed in the forced swim apparatus for 5 minutes. The latency to immobility and total immobility time were used to assess the relative "depressive-like" behavior after global AcbSh or selective AcbSh-LH neuronal activation. Rats were sacked 90 minutes after behavioral testing, and brains were processed with immunohistochemistry to examine levels of cFos (neuronal activation marker) in the AcbSh and LH.
Results: Both global AcbSh stimulation and selective stimulation of the AcbSh-LH output increased "depressive-like" behavior, and AAV-ChR2-EYFP rats had reduced latencies to immobility and increased overall time spent immobile on the FST compared to AAV-EYFP controls. In experiment 1, optogenetic stimulation of cell bodies in the AcbSh increased cFos levels in this region and also reduced cFos levels in the LH, suggesting that optogenetic stimulation in the AcbSh activated GABAergic AcbSh neurons leading to inhibition of target neurons in the LH. In experiment 2, optogenetic stimulation of AcbSh nerve terminals in the LH also reduced cFos levels in the LH, but failed to alter cFos levels in the AcbSh, suggesting that antidromic activation of these neurons did not occur, but rather the behavioral effects were due to local neurotransmitter release from AcbSh nerve terminals in the LH.
Conclusions: Our findings demonstrate that the AcbSh-LH pathway has an important role in mediating mood-related behavior, and that selective stimulation of AcbSh outputs to the LH facilitates "depressive-like" behavior. These results are consistent with previous studies suggesting that "depressive-like" behavior is associated with reduced activity of LH neurons, and our findings further suggest that increased activity of GABAergic AcbSh outputs to the LH may mediate this effect. Our results also suggest that aberrant activity of the AcbSh-LH pathway may facilitate the onset of negative mood states, which may have implications for various psychiatric disorders with a mood dysfunction component (e.g., depression, addiction).
Keywords: depression, nucleus accumbens, lateral hypothalamus, optogenetics, cFos
Disclosure: E. Larson, Nothing to Disclose; D. Self, Nothing to Disclose.

M202. The Localization of the Excitatory Amino Acid Transporter EAAT2 in Prefrontal Cortex in Schizophrenia: A Postmortem Ultrastructural Study
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Background: Schizophrenia is a major mental illness with complex pathology, including abnormalities in the glutamate system. The process of glutamate release, activity, and reclamation involves the astrocyte, the presynaptic neuron and the postsynaptic neuron. Glutamate is rapidly removed from the synapse by a family of plasma membrane excitatory amino acid transporters (EAATs), localized to neurons and astrocytes. To prevent glutamate spillover, EAATs must be expressed at high levels on the astrocytic plasma membrane and be localized adjacent to the synapse. Here we tested the hypothesis that perisynaptic localization of EAAT2 is diminished in schizophrenia, as indicated by an increase in the distance between the synapse and the localization of EAAT2 expression on the astrocytic plasma membrane.
Methods: Postmortem cortical tissue (BA9) was obtained from the Maryland and Alabama Brain Collections from 6 normal controls (NCs) and 7 subjects with schizophrenia (SZ). Age, PMI and pH were 40.5 ± 11.0 yrs, 5.5 ± 1.4 hrs, and 6.9 ± 0.3 for NCs and 48.7 ± 9.4 yrs, 5.4 ± 1.7hrs and 6.2 ± 1.2 for SZ cases. The tissue was processed for immunohistochemistry (IHC) using a primary antibody against EAAT2 (Millipore, AB# 1783), the Elite ABC kit and diaminobenzidine. Layer II was excised and thin sectioned. Pictures were taken at 15,000 magnification in areas where immunocytochemical reaction was optimal. Synapses and adjacent labeled astrocytes were identified and quantified.
Results: EAAT2 labeling was present throughout the grey and white matter in small cells which are most likely glial cells and their processes. Electron microscopic examination of layer III showed EAAT2 labeling in small astocytic processes adjacent to axon terminals forming asymmetric (glutamatergic) synapses. The proportion of asymmetric synapses that were adjacent to labeled astrocytic process was similar between groups (55-57%). However, the mean distance of EAAT2 labeling in perisynaptic astrocytic processes to the edges of asymmetric synapses was significantly ($p < 0.001$) longer in SZ cases (0.421 ± 0.06 μm) than in NCs (0.272 ± 0.05 μm).
Conclusions: The observation that EAAT2 labeled astrocytic processes were significan'ty further away from the synapse in schizophrenia compared to controls is important because such a change could lead to glutamate spillover from the synaptic cleft, activation of extrasynaptic glutamate receptors, as well as loss of input specificity in limbic circuits.
Keywords: glutamate, neuroanatomy, pathology, electron microscopy
Disclosure: R. Roberts, Nothing to Disclose; J. Roche, Nothing to Disclose; R. McCullumsmith, Nothing to Disclose.

M203. Within Subject Evaluation of the Effect of Neuroleptics on GABA Levels in Patients with Schizophrenia Spectrum Psychosis

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Background: γ-amino-butyric acid (GABA) abnormalities are thought to play an important role in the pathophysiology of schizophrenia. GABA levels measured *in vivo* with magnetic resonance spectroscopy (MRS) have been found to be decreased (occipital cortex, basal ganglia), increased (medial prefrontal and parietal cortices) or unchanged (dorsolateral prefrontal cortex) in schizophrenia, but the vast majority of patients studied were chronically treated. The effects of neuroleptics on GABA levels are poorly understood. One study reported a negative relationship between chlorpromazine equivalents and GABA levels, while another reported that GABA levels did not change in first episode patients after 6 months of neuroleptic treatment. Recently, Kegeles et al. (Arch. Gen. Psych. 2012) reported that untreated patients had increased GABA levels in medial prefrontal cortex (MPFC), while treated patients did not. In this study, we compared GABA levels in the MPFC in patients with schizophrenia spectrum disorders before and after discontinuation of a single neuroleptic for at least two weeks.
Methods: Fourteen patients with psychosis (11 with schizophrenia, 1 with schizoaffective disorder, 2 with psychosis NOS; 9 males, 5 females; mean age 27.9 ± 9.5 years) were studied on the NIMH inpatient unit. Subjects were scanned twice (46.4 ± 16.8 days in between scans), once on a single neuroleptic and once off all psychotropic medications. The time off medications was at least 15 days (18.7 ± 4.4). Nine patients were scanned on medications first and then off, the rest in the reverse order. GABA was measured in MPFC by J-edited, single-voxel spectroscopy at 3T (GE scanner) and expressed both as a ratio of GABA to Creatine (Cre) and as a ratio to the water signal corrected for gray, white and CSF content. A paired t-test was used to compare GABA levels on and off neuroleptics.
Results: No statistically significant effect of discontinuation of neuroleptics for at least two weeks on GABA levels (GABA/Cre ON: 0.0987 ± 0.00927 vs. OFF: 0.09804 ± 0.007023 or GABA/Water ON: 1.61241 ± 0.12290 vs OFF: 1.56413 ± 0.14299, institutional units*10^{-3}) was found in 14 patients with psychosis.
Conclusions: Although this is a small sample size, and the neuroleptic withdrawal was short lived, nevertheless the within-subject design is inherently more powerful in detecting medication related changes than across-subjects designs. Many possible confounds need to be taken into account in order to adequately interpret the data: age, sex, narrow vs. broad definitions of schizophrenia, prior history of substance abuse, severity of symptoms, order of scans, neuroleptic dose and type, time between scans, smoking, and menstrual cycle could all influence baseline GABA values or the rate of change of GABA. None of these appeared to be related to the degree of change of GABA levels in preliminary analyses. The data by Kegeles et al. would suggest that GABA levels are elevated in the MPFC of patients with schizophrenia and that neurolpetic treatment normalizes GABA concentrations. Our data do not support this interpretation, however two weeks of drug discontinuation may be too short a time to detect an effect. Indeed, the untreated patients studied by Kegeles et al. were either medication naïve or had been off neuroleptics for much longer intervals than we were able to do here (one was medication free for 14 days, while all the others had been off neuroleptics for periods of one month to four years). Further research with longer drug free intervals and larger samples with a within-subjects design is necessary to adequately assess the effects of neuroleptics on GABA levels in schizophrenia.

Keywords: GABA, magnetic resonance spectroscopy, schizophrenia, neuroleptics, within-subject design
Disclosure: S. Marenco, Nothing to Disclose; K. DeJong, Nothing to Disclose; J. van der Veen, Nothing to Disclose; A. Barnett, Nothing to Disclose; J. Apud, Nothing to Disclose; K. Berman, Nothing to Disclose; D. Weinberger, Nothing to Disclose.

M204. Genetic Background Regulates the Effect of the Antidepressant Fluoxetine on Behavioral Despair and Hippocampal Neurogenesis in Mice

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Background: There is strong evidence that chronic treatment with antidepressants such as fluoxetine induces an increase in adult hippocampal cell proliferation and neuronal differentiation, and that this effect may be associated with the behavioral response to antidepressants.
Methods: In order to test the association between antidepressant efficacy and hippocampal neurogenesis, we treated mice from 30 inbred strains with chronic oral fluoxetine and measured the effect of drug treatment on behavioral despair (tail suspension test) and hippocampal gene expression in all 30 strains. The effect of fluoxetine on neurogenesis (BrdU labeling) was measured in a subset of the 30 strains.
Results: We found that approximately 60% of the strains showed a positive behavioral response to fluoxetine treatment, similar to the percent response observed in human cohorts. Gene expression analysis identified a set of approximately 100 genes, many of which have been associated with neurogenesis, that clustered based on the strain-specific behavioral response to fluoxetine. This gene set was found to reliably predict the effect of fluoxetine on cell proliferation in the dentate gyrus of a subset of the inbred strains. Additional haplotype association mapping identified several genetic loci associated with both the behavioral and neurogenic response to fluoxetine.
Conclusions: These results suggest that the behavioral response to fluoxetine is under genetic regulation and associated with hippocampal neurogenesis: strains that show a positive behavioral response to fluoxetine also show an increase in hippocampal neurogenesis, whereas no change in neurogenesis is observed in strains that do not show a behavioral response. Additional genetic and genomic analysis identified gene networks and genomic loci that may regulate antidepressant efficacy.
Keywords: mouse antidepressant neurogenesis gene expression haplotype mapping
Disclosure: B. Miller, Nothing to Disclose; Z. Zeier, Nothing to Disclose; T. Lanz, **Part 1:** Employee of Pfizer Global Research, **Part 2:** Pfizer Global Research, **Part 3:** Employee of Pfizer Global Research; M. Lopez-Teledono, Nothing to Disclose; M. Pletcher, **Part 1:** Employee of Pfizer Global Research, **Part 2:** Pfizer Global Research, **Part 3:** Employee of Pfizer Global Research; R. Kleiman, **Part 1:** Former employee of Pfizer Global Research, Employee of Selventa, **Part 2:** Pfizer Global Research, Selventa, **Part 3:** Former employee of Pfizer Global Research, Employee of Selventa; C. Wahlestedt, **Part 1:** Epigenetix, OPKO-Curna, Pfizer Global Research, **Part 2:** University of Miami Miller School of Medicine, OPKO-Curna, **Part 4:** Pfizer Global Research.

M205. Functional Genetic Variants in the Vesicular Monoamine Transporter 1 (VMAT1) Modulate Emotion Processing

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Background: Emotional behavior is in part heritable and often disrupted in psychopathology. Identification of specific genetic variants that drive this heritability may provide important new insight into molecular and neurobiological mechanisms involved in emotionality. Recently genetic variants in the vesicular monoamine transporter 1 (*VMAT1*) have been associated with bipolar disorder/schizophrenia, disorders that are characterized by abnormal emotionality. Here, we used a translational research approach to investigate the neural mechanism underlying emotional behavior modulated by variation in the presynaptic *VMAT1* gene using a stepwise approach beginning with identification of a functional locus, namely a common missense variants, and tracing effects of this variant on behavior via two fMRI paradigms in independent samples.
Methods: VMAT1 full length cDNA was isolated from post-mortem human substantia nigra tissue. Site-directed mutagenesis for Thr4Pro, Ser98Thr and Thr136Ile and their common haplotypes was carried out, CV-1 cell lines were transfected with constructs and monoamine neurotransmitter uptake was determined using standard methods. Functional imaging data and genotypes for the above variants were obtained for 102 subjects that performed an affective word task during which they silently read emotionally valenced words. BOLD signal was measured in the whole brain using a 3-Telsa scanner. Percent signal change in the medial prefrontal cortex (mPFC) in response to negative words was compared between genotype groups. To further explore the effects of the Thr136Ile polymorphism on emotion processing, we examined whether Thr136Ile genotype is associated with individual differences in amygdala reactivity to threat. Genotype and neuroimaging data were available from 298 participants who completed the Duke Neurogenetics Study.
Results: *In vitro* experiments show that the 136Ile variant leads to significantly increased transporter function for dopamine, serotonin and norepinephrine (uptake Thr136 versus Ile136 $p < 0.001$). *In vivo* imaging experiments document that Thr136 homozygotes ($n = 63$) showed greater responses to negative words than Ile136 carriers ($n = 39$) ($p = 0.008$, $F_{1,96} = 7.2$) in a single region of interest in the medial prefrontal cortex. Regression analyses in the amygdala reactivity imaging dataset revealed a significant effect of Thr136Ile genotype on the overall magnitude of threat-related amygdala reactivity (overall model: $F(7,290) = 2.87$, $p = 0.006$).
Conclusions: Our results demonstrate that the presynaptic vesicular monoamine transporter 1 (*VMAT1*) Thr136Ile (rs1390938) polymorphism is functional *in vitro*, with the Ile allele leading to increased monoamine transport into presynaptic vesicles. Moreover, we show that the Thr136Ile variant predicts differential responses in emotional brain circuits consistent with its effects *in vitro*. Taken together, our data show that *VMAT1* polymorphisms influence monoamine signaling, the functional response of emotional brain circuits, and might contribute to risk for psychopathology.
Keywords: emotion, amygdala, prefrontal cortex, genetics
Disclosure: F. Lohoff, Nothing to Disclose; B. Mickey, Nothing to Disclose; M. Heitzeg, Nothing to Disclose; S. Langenecker, Nothing to Disclose; J. Zubieta, **Part 1:** Eli Lilly & Co., Johnson & Johnson, Merck, and Abbott; R. Bogdan, Nothing to Disclose; Y. Nikolova, Nothing to Disclose; A. Hariri, Nothing to Disclose; L. Bevilacqua, Nothing to Disclose; D. Goldman, Nothing to Disclose; G. Doyle, Nothing to Disclose.

M206. Expression of Histone-modifying Genes in Response to Cocaine

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Background: Chronic cocaine use causes long-lasting changes in gene expression in the brain's reward areas that contribute to persistent drug-seeking and drug-taking behaviors. Recent reports indicate that histone-modifying proteins are key regulators in such neuroadaptations and behaviors. Thus, understanding epigenetic factors associated with addiction is crucial to develop novel therapeutic interventions. However, of the hundreds of epigenetic-related proteins known to exist, only a small fraction have been studied in the context of addiction. Here, we investigated the expression of numerous histone-modifying genes in the nucleus accumbens following chronic cocaine injections or self-administration.
Methods: Male Sprague Dawley rats received 10 days of chronic intraperitoneal injections of saline or cocaine (one 10 mg/kg injection per day) or 10 sessions of intravenous cocaine self-administration (2 hr session a day, 0.2 mg/infusion). Using a Nanostring panel (a method for measuring RNA expression level of many genes, comparable to RT-PCR), expression of 122 histone-modifying genes were measured in the nucleus accumbens 24 hours after the last drug exposure.
Results: Of the histone-modifying genes that were measured, 2.8% were down-regulated when rats received cocaine injections, compared to saline-injected rats. Interestingly, none of the genes were up-regulated. In animals that self-administered cocaine, 14% and 9% of genes were significantly up- and down-regulated, respectively compared to animals that received experimenter-administered cocaine injections of a similar dose.
Conclusions: In the current study, several unexplored histone-modifying genes were altered in the nucleus accumbens following cocaine self-administration and/or non-contingent injections of cocaine. Studies in our laboratory aimed at determining whether specific histone-modifying genes play an important role in drug seeking and self-administration of cocaine are ongoing. Results from these studies may shed light on novel epigenetic processes involved in cocaine intake, which could ultimately lead to new therapeutic avenues for drug addiction.
Keywords: cocaine, epigenetics, self-administration, histone-modifying genes
Disclosure: G. Sartor, Nothing to Disclose; S. Brothers, Nothing to Disclose; S. Izenwasser, Nothing to Disclose; C. Wahlestedt, **Part 4:** I have a financial interest in Opko Health biotechnology company. These experiments, however, are not related to this financial involvement.

M207. Glutamatergic Hyperexcitability during Alcohol Withdrawal-precipitated Aggression in Mice

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Background: Ethanol withdrawal symptoms are one important feature of alcohol dependence. Similarly, caretakers of alcoholics have reported heightened irritability and aggression during ethanol withdrawal. Much research implicates glutamate hyperexcitability and an upregulation of NMDA receptors as one target for studying the neurobiology of ethanol withdrawal. The present study examined the effects of the uncompetitive NMDA receptor antagonist memantine on aggression during ethanol withdrawal.
Methods: Outbred Carworth Farm Webster (CFW) and inbred C57BL/6J (B6) male mice were given intermittent, 2-bottle choice

to 20% w/v ethanol and concurrently to water for 10 weeks. To measure alcohol withdrawal severity after 8 weeks of escalated drinking, handling-induced convulsion scores were assessed every two hours from hours 0-10 during the ethanol withdrawal period. Individuals were also challenged with memantine or ketamine to assess glutamate excitability during withdrawal aggression. Resident CFWs were injected with memantine (0, 3, 5, 10, or 30 mg/kg, i.p.) or ketamine (0, 3, 5, or 10 mg/kg, i.p.) and tested for aggression against an intruder at eight hours into withdrawal. Resident B6 s were also tested for withdrawal-related aggression with memantine (0, 1, 3, 5, 10 mg/kg, i.p.) and ketamine. Additional groups of mice were given access to water for 10 weeks as contemporary controls. Lastly, extracellular glutamate in the prefrontal cortex (PFC) was measured at eight hours into withdrawal from 10 weeks of escalated alcohol drinking to verify if glutamate is increased by a memantine injection.

Results: CFW males voluntarily consumed ethanol, ranging from 5-20 grams/kilogram (g/kg) bodyweight in 24 hours while B6 males consumed ethanol greater than 20 g/kg/24 h consistently over the 8 weeks of intermittent access. B6 mice given intermittent access to ethanol had greater convulsion severity than CFWs. Both alcohol drinking groups had greater seizures than water drinking animals. Memantine significantly increased aggression in CFW mice during the withdrawal period, specifically at the 5 mg/kg dose. On the other hand, memantine dose-dependently reduced aggression in B6 mice. Ketamine (1-10 mg/kg) had no effect on withdrawal aggression in either strain. Preliminary microdialysis results indicate that PFC extracellular glutamate is elevated during peak ethanol withdrawal, and this increase may be attenuated by memantine injection.

Conclusions: These findings suggest that the uncompetitive NMDA receptor antagonist memantine, but not ketamine, biphasically increased withdrawal-related aggression in outbred CFW mice, but dose-dependently reduced aggression in high ethanol drinking B6 mice. There may be significant strain differences in NMDAR expression. The current microdialysis data suggest that memantine antagonizes the inhibition of aggressive behavior during ethanol withdrawal, thus leading to increased aggression during that period. Ongoing microdialysis studies continue to compare extracellular glutamate levels in these two strains to further characterize the hyperglutamatergic state as well as the development of ethanol withdrawal over time.

Keywords: ethanol withdrawal, aggression, glutamate, memantine

Disclosure: L. Hwa, Nothing to Disclose; A. Nathanson, Nothing to Disclose; K. Dodman, Nothing to Disclose; A. Shimamoto, Nothing to Disclose; J. DeBold, Nothing to Disclose; K. Miczek, Nothing to Disclose.

M208. Opioid Modulation of Marijuana's Analgesic, Subjective, Reinforcing, and Physiological Effects in Non-treatment Seeking Marijuana Smokers

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Background: Preclinical and clinical studies demonstrate that opioids modulate the effects of cannabinoids. We recently demonstrated that the opioid receptor antagonist naltrexone increased the intoxicating effects of marijuana in non-treatment seeking marijuana smokers (Cooper and Haney, 2010). Marijuana also reduced the response to pain in the Cold-Pressor Test (CPT), a laboratory model of pain (Cooper et al., in preparation). However it remains unknown whether opioids also modify this cannabinoid effect. In order to clarify opioid modulation across distinct cannabinoid endpoints, the objective of this double-blind, placebo-controlled study was to directly assess the effects of an opioid agonist and antagonist on marijuana's pain-relieving, intoxicating, reinforcing, and physiological effects.

Methods: Non-treatment seeking marijuana smokers participated in this 8-session outpatient study during which the pain-relieving, subjective, reinforcing (i.e., self-administration), and physiological (mydriatic, cardiovascular) effects of smoked marijuana were evaluated. In each session, participants smoked 75% of an inactive (0.0% THC) or active (5.6% THC) marijuana cigarette 45 minutes after ingesting capsules containing placebo, naltrexone (25 mg, PO) or sub-analgesic doses of the opioid agonist, oxycodone (2.5 or 5.0 mg, PO). Analgesic, subjective, mydriatic, and cardiovascular effects were measured at various time points throughout each 6-hour session. During the CPT, participants immersed their hand in cold water (4°C) for up to three minutes, and the times at which the participant first reported pain (pain sensitivity) and withdrew the hand from the water (pain tolerance) were recorded. Subjective pain ratings were also measured immediately after each CPT. In the afternoon, participants were given the opportunity to purchase 1, 2, or 3 puffs of the marijuana smoked earlier in the session using their study earnings.

Results: Preliminary analysis was completed on the 5 study completers to date (4M; 1F) who smoked an average of 11.8 ± 2.6 marijuana cigarettes per day, 7 ± 0.2 days per week. No differences between inactive and active marijuana were observed in subjective pain ratings or pain response in the CPT. Relative to placebo, the higher dose of oxycodone (5.0 mg) increased pain tolerance when active marijuana was smoked ($p \leq 0.05$), and a trend for decreased pain sensitivity was observed under low-dose oxycodone conditions ($p \leq 0.10$). Relative to inactive marijuana, active marijuana increased ratings of positive subjective drug effects including 'High,' Liking,' and ' Good Effect' ($p \leq 0.01$). Naltrexone and oxycodone did not affect these ratings. Active marijuana also increased heart rate relative to inactive marijuana ($p \leq 0.05$); naltrexone and oxycodone did not affect this endpoint. Pupil diameter increased when active marijuana was smoked relative to inactive marijuana ($p \leq 0.05$); naltrexone and both doses of oxycodone decreased pupil diameter relative to placebo when administered before active marijuana was smoked ($p \leq 0.001$). Marijuana self-administration did not vary according to marijuana or drug condition.

Conclusions: These preliminary results suggest that sub-analgesic doses of oxycodone selectively increased the analgesic effects of marijuana while producing no enhancement of intoxication. Both naltrexone and oxycodone attenuated marijuana-induced mydriasis. Ongoing recruitment will facilitate further assessment of the interactions between marijuana and naltrexone and oxycodone and will help to elucidate the contribution of opioids to cannabinoid-induced analgesia and abuse liability while providing information regarding the potential therapeutic use of opioid-cannabinoid combinations for the treatment of pain.

Keywords: Cannbinoids, opioids, marijuana, oxycodone, naltrexone, pain, analgesia, self-administration, abuse liability

Disclosure: Z. Cooper, Nothing to Disclose; S. Comer, Nothing to Disclose; G. Bedi, Nothing to Disclose; D. Ramesh, Nothing to Disclose; M. Haney, Nothing to Disclose.

M209. Rostral vs. Caudal Anterior Cingulate Connectivities Differentiate Two Neurotherapuetic Targets Used for OCD and MDD

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Background: The pathophysiology of obsessive-compulsive disorder (OCD) and major depressive disorder (MDD) remain unknown, but converging lines of evidence point to abnormalities in the prefrontal cortex. Research has focused on the ventromedial prefrontal and orbitofrontal cortices, but neuroimaging results

also emphasize the importance of the dorsal anterior cingulate cortex (dACC) in these disorders. The dACC is structurally and functionally abnormal in OCD and MDD and may be crucial for the effectiveness of neurosurgical interventions for treatment-resistant OCD and MDD. Cingulotomy ablates dACC white and grey matter, yet little is known about the network interrupted by this lesion. Likewise, anterior internal capsule (AIC) ablation (capsulotomy) or stimulation (deep brain stimulation—DBS) is also an effective treatment for OCD and MDD and involves dACC projections through the AIC. However, the location of these fibers within the AIC and their subsequent terminations remain unclear. Understanding dACC circuits will help define the neural networks associated with OCD and MDD, and thus help to refine existing neurosurgical treatments and suggest novel, less invasive therapeutics. These experiments were designed to delineate pathways and terminations of dACC fibers through the cingulum bundle and the AIC to predict the neural circuitry impacted by each therapeutic site.

Methods: We injected bidirectional tracers into different functional regions of the dACC of adult macaque monkeys. We mapped the efferent fiber pathways and terminals through the cingulum bundle and the AIC, then rendered them in 3-D in a standard macaque brain to determine the dACC projections most likely targeted in cingulotomy, DBS in the AIC, and capsulotomy. The injection sites were located at approximately the site of a typical cingulotomy lesion and rostral to the site. Analysis included comparing injections that were placed within versus outside the region targeted for cingulotomy, and the placement of the DBS electrode.

Results: With a 3-D rendering, we were able to visualize projection pathways and compare across different injection sites. As expected, we found substantial fiber projections from dACC through both neurotherapeutic sites. Fibers entered the cingulum bundle immediately from the injection site. Within the cingulotomy site, there were substantial fibers present from adjacent dACC gray matter, but also fibers traveling caudally within the cingulum bundle from other dACC locations. Thus, despite well-placed lesions, tracts affected by cingulotomy will encompass functionally diverse dACC fibers passing through the site, including pregenual dACC. In addition, terminals from dACC to cingulate and dorsal prefrontal cortex regions were not evenly distributed. In other words, each dACC region formed specific projection zones via the cingulum bundle. These varied according to the rostral-caudal position of the origin. All dACC fibers traveled relatively medially within the AIC, with many coursing through fascicles embedded in the caudate nucleus. Fibers originating rostrally within the dACC traveled ventral in the AIC to those originating more caudally. Because of this organizational principle, the modeled DBS electrode for OCD contacted far more rostral dACC fibers than caudal ones. This contrasts with the cingulotomy site, which is likely to have a greater impact on caudal dACC. At the level of the anterior commissure, fibers split into three branches: one branch terminated in thalamus, another descended to the pons and cerebellum. The third branch terminated in subcortical regions known to have psychiatric relevance. These included the subthalamic nucleus, another DBS target for OCD, and the raphe nuclei, the source of the brain's serotonin. Preliminary analyses indicate that rostral dACC, relative to caudal, projected more strongly to raphe.

Conclusions: We examined the projections of dACC fibers through two surgical targets, the cingulum bundle and the AIC. These sites focus on overlapping but distinct connections. The cingulotomy target involves both cortico-cortical and cortico-subcortical pathways, whereas the AIC region targets cortico-subcortical pathways. While both sites affect all of dACC, the AIC target impacts more on the rostral dACC, while the cingulotomy target impacts more on the caudal dACC. Our observations indicate that more posterior cingulotomy lesions will capture rostral dACC cortico-cortical fibers coursing caudally, but more anterior lesions may not capture an equivalent set of caudal dACC fibers. Therefore, the more caudal lesions will encompass a greater portion of the dACC cortico-cortical fibers. Likewise, rostral and caudal dACC may have different subcortical projection densities, particularly to the brain's serotonergic system. Our results demonstrate the unique connections of rostral and caudal dACC. These connectivities likely differentially impact on the two neurosurgical targets. The dACC also shows rostral/caudal functional distinctions, with rostral areas more associated with emotion and caudal ones linked with cognition. Taken together, these studies indicate a more rostral site may be a key node in the network associated with OCD and MDD as well as a potential neurotherapeutic target.

Keywords: cingulotomy; MDD; OCD; DBS; connectivity

Disclosure: S. Heilbronner, Nothing to Disclose; S. Haber, **Part 1:** Dr. Haber has received consultation fees from Medtronic, Inc and Pfizer, Inc.

M210. Experimental Sadness Induces Plasma IL-18 Elevation and Covarying Modulation of Limbic Endogenous Opioid Function in Major Depression

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Background: Existing evidence suggests that depressive symptoms are associated with reduced health status and predisposition to frequently co-morbid medical illnesses. Increased research efforts have yet to decipher the pathophysiologic mechanisms underlying the propensity for psychosomatic comorbidity. Evidence shows elevated interleukin-18 (IL-18), a pro-inflammatory cytokine structurally homologous to IL-1β, to be associated with both depression and specific medical syndromes highly co-morbid with MDD (i.e. pain states, heart disease, metabolic syndrome, persistent pain). Additionally, a well-known stress regulating central neurotransmitter system, the endogenous opioid (and its μ-opioid receptors), has been shown dysregulated in stress-related pathological states (i.e. depression, acute and chronic pain). We have previously reported that IL-18 plasma levels at baseline were correlated with regional μ-opioid receptor availability in MDD volunteers. Here we examined directly the impact of experimentally induced sustained sadness on plasma levels of IL-18 and their relationship with changes in central endogenous opioid neurotransmission.

Methods: Using the Velten technique, we induced sad (and neutral) affective states over 30 min in 28 female volunteers (13 unmedicated MDD and 15 healthy control volunteers) to test if experimentally induced sadness increases IL-18 concentration, a potential mechanistic factor underlying psychosomatic co-morbidities. Further, we hypothesized that sadness-induced limbic μ-opioid receptor activation (e.g., amygdala, ventral pallidum) would be directly proportional to sadness induced IL-18 elevation. Plasma samples were obtained from whole blood prior to neuroimaging and following each mood induction (i.e. both sad and neutral) within the positron emission tomography (PET) neuroimaging scan (i.e. 45 min, 90 min), volunteers being randomized and counterbalanced to induction order. IL-18 concentrations were determined via standardized ELISA techniques following manufacturer instructions (Millipore, MN). Using PET and the μ-opioid receptor selective radiotracer [11C]Carfentanil, we obtained measures of both μ-opioid receptor availability (i.e. non displaceable binding potential, BP_{ND}) during a neutral mood state and the difference in BP_{ND} between sad and neutral moods, a measure of changes in endogenous opioid neurotransmitter release. ANCOVA was utilized to examine the effect of affective state on IL-18, μ-opioid receptor availability and endogenous opioid neurotransmitter release on a voxel-by-voxel basis using SPM8 (Statistical

Parametric Mapping, Wellcome Trust, London, UK), brain images normalized to the MNI (Montreal Neurological Institute, Montreal, Canada) template. Significance within neuroimaging analyses was detected using an uncorrected statistical threshold of $p < 0.0001$ for previously hypothesized regions (rostral anterior cingulate, nucleus accumbens, ventral pallidum, amygdala, medial and posterior thalamus, hypothalamus). Statistical significance for other analyses was calculated using a statistical threshold that controls a Type-I error rate at $p = 0.05$.

Results: Our results show experimental mood induction to significantly effect IL-18 ($F = 11.7$, $p < 0.001$), sadness increasing IL-18 ($T = -2.7$; $p = 0.01$) and neutral mood induction reducing IL-18 below both baseline ($T = -4.0$; $p < 0.01$) and sadness ($T = -2.7$; $p = 0.01$). The effect of sadness on IL-18 differed between diagnostic groups ($F = 4.5$, $p = 0.02$) being greater in MDDs ($F = 11.9$, $p = 0.002$) than in controls ($F = 1.7$, $p = 0.22$). Sadness-induced increases in IL-18 were directly proportional to the sadness-induced μ-opioid system activation in the amygdala bilaterally, ventral tegmental area, hypothalamus, left ventral pallidum and medial thalamus bilaterally in MDD patients. In controls, similar relationships were observed in the right hypothalamus.

Conclusions: These data demonstrate dynamic changes of a pro-inflammatory IL-1 superfamily cytokine, IL-18, and its relationship to μ-opioid neurotransmission in response to experimentally induced sadness, providing preliminary evidence for IL-18's mechanistic involvement in the psycho-somatic translation of negative affective states. These changes in IL-18 induced by a sustained sadness challenge and their relationship with central μ-opioid neurotransmission were particularly pronounced in MDD volunteers. These findings provide further evidence to implicate IL-18 both in the pathophysiology of MDD and potentially as a factor predisposing these individuals to medical co-morbidities associated with elevations in pro-inflammatory cytokines (i.e. diabetes, heart disease, persistent pain states).

Keywords: psychoneuroimmunology depression IL-18 cytokine PET neuroimaging carfentanil endogenous μ-opioid receptors

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M211. Long-term Potentiation of Visual Stimulus-induced EEG Phase Synchrony: Further Evidence for Impaired Visual Cortical Plasticity in Schizophrenia

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Background: Long-term potentiation (LTP), a basic mechanism of synaptic plasticity thought to underlie learning and memory, depends on glutamatergic neurotransmission at NMDA receptors. Converging evidence implicates NMDA hypofunction in schizophrenia and its associated cognitive impairments, suggesting that disruption of basic mechanisms of synaptic plasticity, including LTP, may be a core feature of the disorder's pathophysiology. Classic LTP paradigms involving stimulation at a "tetanizing" frequency have been successfully adapted for *in vivo* studies of humans, showing LTP-like potentiation of visual evoked potentials (VEPs) from scalp-recorded electroencephalography (EEG) following repeated rapid visual stimulation. Recently, we showed that this LTP-like potentiation of VEPs following exposure to a photic tetanus is deficient in schizophrenia. Here, we extend this prior work by examining the effect of photic tetanization on the stimulus-locked inter-trial phase synchrony of EEG oscillations in schizophrenia patients (SZ) and healthy controls (HC).

Methods: EEG was recorded from 19 medicated SZ patients and 23 HC during a visual LTP paradigm. Using Morlet wavelet decomposition of single trial EEG epochs, we calculated the phase-locking factor (PLF) for theta, beta and gamma band oscillations induced by a visual stimulus (checkerboard) at baseline, and at 2, 4 and 18 minutes following a 2-minute photic tetanus (same checkerboard flashing at 9.83 Hz frequency).

Results: The photic tetanus produced significant increases (i.e., potentiation) of theta, beta, and gamma phase synchrony (PLF) at 2, 4, and 18 minutes post-tetanus, relative to baseline. The potentiations of theta and beta phase synchrony were significantly attenuated in SZ, relative to HC, whereas potentiation of gamma synchrony did not differentiate the groups.

Conclusions: Exposure to a photic tetanus not only induces lasting increases in the amplitudes of VEPs to the tetanizing stimulus, it also induces enhancements in stimulus-locked phase synchrony of theta, beta, and gamma oscillations. SZ patients show deficiencies in the potentiation of both VEP amplitudes and phase synchronizations of theta and beta oscillations, consistent with hypothesized deficits in synaptic plasticity putatively related to NMDA-receptor hypofunction.

Keywords: Schizophrenia, EEG, plasticity, oscillations, Long-term potentiation

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M212. The Functional Contribution of Distal Amygdala Projections in Anxiety: An Optogenetic Circuit Dissection

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Background: Anxiety disorders have the highest prevalence of all diagnosed psychiatric diseases (28% lifetime) in the United States. Understanding the underlying neural circuitry of anxiety is integral to understanding what causes this evolutionarily hard-wired response to become maladaptive. Previously, we showed that selectively activating a projection from the basolateral amygdala (BLA) to the central nucleus of the amygdala (CeA) produced a robust anxiolytic effect while activating BLA somata produced an anxiogenic effect. We hypothesized that this was because BLA projections to different downstream targets could produce distinct behavioral phenotypes.
Methods: To dissect the neural circuits underlying anxiety, we use optogenetic projection-specific techniques. Using these genetically-encodable, light-sensitive proteins allows us to control the activity of specific neurons and neuronal projections with unprecedented precision. To test our hypothesis, we wanted to explore BLA projections to other distal targets.
Results: Indeed, through the exploration of BLA projections to other targets that have been implicated in anxiety-related behaviors or anxiety in humans, such as the ventral hippocampus, we identified that some projections from the BLA produce anxiogenic behavioral phenotypes.
Conclusions: We therefore conclude that BLA projections to different downstream targets produces distinct behavioral phenotypes. This offers an explanation for our previously reported finding that activating BLA somata could have a net effect that was different from BLA-CeA projections.
Keywords: amygdala anxiety optogenetics projection-specific ventral hippocampus
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M213. Oxytocin Associated with More Negative Evaluation of Neutral Faces after an Affective Learning "Gossip" Task for Men Compared to Women

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Background: Although prior data suggest that oxytocin might have prosocial effects by decreasing the negative affective evaluation of social faces after negative associations are learned through aversive conditioning (Petrovic et al., 2008), little is known about the nature of oxytocin's effects on affective associative learning. Further, while results from recent research in women (Domes et al., 2010) suggest that oxytocin might exert differential effects across genders, most studies conducted to date have been in men. The present study aimed to examine whether administration of oxytocin vs. placebo may influence affective associative learning in a task in which neutral faces were paired with emotionally-valenced written phrases ("gossip"). Based on the prosocial nature of oxytocin, non-psychiatrically ill individuals receiving oxytocin were hypothesized to exhibit less negative affective associative learning than individuals receiving placebo. In addition, gender was examined as a moderating factor in this relationship.
Methods: Healthy controls free of psychiatric disorders per clinical interview with the Structured Clinical Interview for DSM-IV (First et al., 2002) were consented to participate in a randomized double-blind administration of intranasal oxytocin or placebo prior to computer based tasks at the Massachusetts General Hospital. Pregnancy, lactation and concurrent use of psychotropic medication or hormones were also exclusionary. Thirty IU of double-blind intranasal oxytocin (n = 25, mean (SD) age = 42.2(11.6), 40% women) or placebo spray (n = 22, mean (SD) age = 44.5(9.6), 36.4% women) was administered 30 minutes before the tasks. Participants underwent an affective learning task consisting of a learning phase and a test phase. During the learning phase, participants viewed 30 neutral faces, each paired with one sentence (gossip) describing a negative behavior, a positive behavior, or a neutral behavior and were told to imagine each target person performing the behavior described in the corresponding piece of gossip (Bliss-Moreau et al., 2008). Face-gossip pairings were displayed for 5 s each in random order within four blocks counterbalanced across participants, so that each face sentence pair was seen four times during the learning phase. During the test phase, the 30 neutral faces from the learning phase plus an additional 10 novel neutral faces were presented. Participants were instructed to rate (with a quick "snap" judgment) each face on a 3-point scale (negative = −1, neutral = 0or positive = 1) using labeled keys on a standard keyboard. A 4x2x2 repeated measure analysis of variance (ANOVA) with the gossip type as the repeated measure (negative, positive, neutral, novel), gender and drug condition as the between participant factors and affective judgments as the dependent variable, was conducted to examine whether oxytocin administration influences affective learning in the gossip task. Further, 2x2 ANOVAs with gender and drug as between-participants factors were conducted as follow-up analyses for each gossip type.
Results: Mean (SE) ratings for positive, neutral, negative, and novel conditions were 0.03(0.06), −0.04(0.05), −0.30(0.04) and -0.12(0.05), respectively. There was a main effect of gossip type, $F(3,129) = 14.20$, $p < .001$, but no main effects of gender, $F(1,43) = .281$, $p = .60$, or drug $F(1,43) = .102$, $p = .75$, or drug X gossip type ($F(3,129) = 1.01$, $p = .393$). Finally, the interaction between gender and drug condition, was significant ($F(1,43) = 8.08$, $p < .01$). Across all gossip types, male participants in the oxytocin group gave more negative ratings than their counterparts in the placebo group (mean (SE) = −.22(.06) vs. −.04(.07)) while female participants in the oxytocin group gave more positive ratings than their counterparts in the placebo group (mean(SE) = .03(.08) vs. −.21(.09)). Results from a series of 2X2 ANOVAs for each gossip type showed a significant drugXgender interaction effect for the positive gossip type ($F(1,43) = 5.59$, $p = 0.023$) and the negative gossip type ($F(1,43) = 7.47$, $p < 0.01$), but also for the neutral gossip type ($F(1,43) = 4.52$, $p = 0.039$).
Conclusions: Gossip pairing influenced how faces were evaluated as shown by a main effect of gossip type, but contrary to our hypothesis, for the full sample, evaluations did not differ for oxytocin compared to placebo. Men receiving oxytocin, however, rated faces more *negatively* after the gossip manipulation than

those on placebo, whereas women rated faces more *positively* with oxytocin compared to placebo. Further examination revealed that the drugXgender interaction was consistent across the three types of gossip (negative, positive, neutral), suggesting that this effect might hold regardless of the valence of the affective learning. One explanation might be that oxytocin, instead of being simply pro-social, might facilitate approach behaviors generally, including both cooperative- and competitive-type approach behaviors. (Kemp and Guastella 2011) For example, other research suggests that oxytocin may enhance defensive aggression toward competing out-groups in men (De Dreu et al., 2010). More research is needed to understand potential gender differences in the effect of oxytocin on social perception.

Keywords: oxytocin, emotion perception, affective learning, gender

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M214. Impaired Cognitive Flexibility Following Single Prolonged Stress is Ameliorated by Pretreatment with D-Cycloserine

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Background: Recent data suggests that D-Cycloserine (DCS) may be effective in facilitating extinction leaning, which may in turn be effective at ameliorating deficits in these processes associated with anxiety disorders such as Post-traumatic stress disorder (PTSD). DCS is a partial agonist at the *N*-methyl-D-aspartate (NMDA) glutamatergic receptor, and facilitated neural transmission in prefrontal cortex (PFC) may be the mechanism by which DCS corrects extinction deficits. PTSD is also associated with other neurocognitive impairments that may be attributable to deficits in function of the PFC, such as cognitive flexibility. Deficits in both extinction retention and cognitive flexibility have been reproduced in the single prolonged stress (SPS) rodent model of PTSD, which also reduces glutamate in PFC. If DCS "corrects" extinction deficits in PTSD models via glutamatergically facilitated neurotransmission in PFC, then it may also be able to correct deficits in cognitive flexibility. In two experiments, we examined the effect of SPS on cognitive flexibility and the ability of DCS to ameliorate this SPS-induced deficit.

Methods: Male Sprague Dawley rats were trained to press retractable levers in an operant chamber, matched for performance and assigned to either SPS or control groups. Following SPS, rats received three additional lever press sessions, followed by a side preference test on the third day. One day later they learned a response discrimination rule (press left or right lever, opposite to side bias) and on a subsequent day were tested for reversal to the opposite lever. First, reversal learning was compared in SPS and control rats ($n = 32$), then in the second experiment ($n = 64$) DCS (15 mg/kg) or vehicle was administered to SPS and control animals, 30 minutes prior to reversal test.

Results: SPS and control rats showed comparable performance on initial rule acquisition [$t(29) = .055$, $p = .80$] but SPS rats made more errors during rule reversal [$F(1,29) = 4.37$, $p < .05$]. In the second experiment, DCS differentially impacted control (unstressed) and SPS rats [stress x treatment interaction [$F(1,60) = 4.1$, $p < .05$], showing no effect on cognitive flexibility in controls but a beneficial impact in SPS rats (fewer trials to reach reversal learning criterion).

Conclusions: These data suggest that SPS induces impairments in cognitive flexibility, and that this impairment may be at least partly ameliorated with DCS pretreatment. They suggest that SPS-induced impairment of reversal of simple stimulus-reward associations may arise from decreased glutamate transmission in regions of PFC, and that DCS effects on PFC glutamate may produce cognitive benefits beyond its proven impact on extinction learning.

Keywords: Post-traumatic stress disorder, reversal learning, glutamate, prefrontal cortex, rat

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