## A proposed mechanism for the reversal of cocaine-induced synaptic plasticity in the VTA

Long-term plasticity at excitatory synapses, such as long-term potentiation (LTP) and long-term depression (LTD), have been extensively studied because of their ability to act as powerful bidirectional modulators of neuronal activity, and could represent key cellular phenomena underlying learning processes in a variety of brain regions.

A series of recent studies have highlighted the potential role of LTP and LTD in the mesolimbic system as molecular events underlying behavioral maladaptations to motivational stimuli, eventually leading to substance abuse. The first study to provide evidence that drugs of abuse lead to long-term changes in synaptic strength showed that a single *in vivo* exposure to cocaine is sufficient to trigger LTP of AMPA receptor (AMPAR)-mediated excitatory inputs onto dopaminergic (DA) cells in the ventral tegmental area (VTA) (Ungless *et al*, 2001); although subsequent studies have confirmed and extended these initial findings, an understanding of the mechanisms underlying these forms of drug-induced neuroplasticity is still lacking.

A recent study by the Luscher group has begun to shed light on this issue (Mameli *et al*, 2007). It indicated that cocaine-potentiated synapses show synaptic insertion of GluR2-lacking receptors and that mGluR-dependent LTD could efficiently reverse this switch in subunits (Bellone and Luscher, 2006). The latest study by the same group provides further details on the mechanisms of VTA mGluR-LTD and proposes that the reversal of cocaine-induced synaptic plasticity is due to replacement of AMPARs by newly synthesized low-conducting GluR2-containing AMPARs (Mameli *et al*, 2007). Interestingly, they show that mGluR-LTD-induced GluR2 insertion depends on *de novo* protein synthesis of the subunit via mGluR1-mediated activation of phosphoinositide 3-kinase-Akt-mammalian target of rapamycin (mTor), a protein kinase that regulates cell growth, cell survival, and protein synthesis. Because the observed increase in GluR2 subunits is very rapid, it might be attributable to local translation of mRNA readily available in dendrites, in agreement with previous studies on other synaptic proteins (eg, Schilstrom *et al*, 2006).

Acute regulation of the GluR2 content of AMPARs has been reported in other brain regions and is functionally relevant, because it determines the changes in amplitude of synaptic currents, calcium permeability, voltage dependence, and facilitation properties. In cerebellar stellate cells (Liu and Cull-Candy, 2000), increased synaptic activity and $Ca^{2+}$ influx can directly determine the subunit composition of AMPARs; native GluR2-lacking AMPARs are rapidly replaced by GluR2-containing $Ca^{2+}$-impermeable receptors as a self-regulating mechanism to further reduce calcium entry.

In CA1 neurons, a proposed role for the insertion of $Ca^{2+}$-permeable GluR2-containing AMPARs is to transiently 'tag' potentiated synapses and initiate intracellular $Ca^{2+}$-dependent pathways that lead to protein synthesis-dependent long-term strengthening of the synapse (Plant *et al*, 2006) before being replaced by GluR2-containing receptor within 30 min. In the VTA, increased levels of GluR2-lacking receptors induced by cocaine persist not for minutes but for several hours, and exchange with GluR2-containing receptors seems to be dependent on synaptic activity, mGluR1 activation, and *de novo* synthesis of the subunit.

In conclusion, the study by Mameli *et al* (2007) is important, as they have identified a form of LTD that is capable of reversing the already established cocaine-induced neuroplasticity in the VTA. In characterizing the underlying mechanism, Mameli's work suggests that modulation of mGluR1 could open new pharmacological avenues aimed at interfering or reversing the context-dependent reward value of cocaine during abstinence.

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## Oscillatory synchrony: insight into the pathophysiology of psychiatric disorders

Processing and transmission of information in brain require defined temporal boundaries, signaling the beginning and end of transmitted messages. Packaging of information into firing patterns of neuronal as-

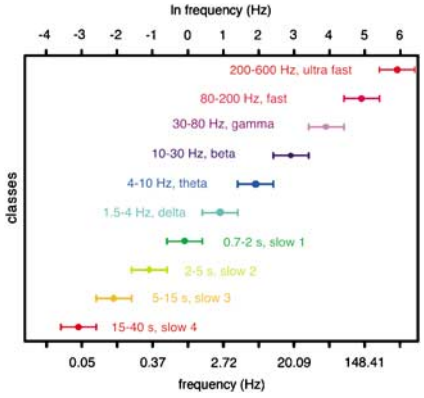


FIGURE. A system of rhythms in the cerebral cortex. Note linear progression of the frequency classes on the ln scale. For each band, the range of frequencies is shown together with their commonly used terms (Buzsáki and Draguhn (2004). Science 304: 1926–1929).

semblies and assembly sequences is often provided by network oscillations. Oscillatory synchrony within and across cortical regions allows for the exchange of meaningful messages. Interference with this critical timing mechanism inevitably results in cognitive impairment.

Irrespective of environmental inputs, cortical networks are in perpetual work (Bressler and Kelso, 2001; Thompson and Varela, 2001) due to the dynamic competition between excitatory and inhibitory forces. This 'spontaneous' or self-organized activity is supported by numerous oscillations that emanate from opposing inhibitory–excitatory actions in local networks or extended thalamocortical territories (Llinás and Steriade, 2006). Because of the limited propagation speed of action potentials in axons of central neurons, fast oscillations typically involve a small volume of neurons, whereas slower oscillations can recruit cell populations of increasing sizes. Although we have known about brain oscillations for a long time, it is only recently that we have begun to explore and understand how brain rhythms assist the formation of cell assemblies in support of cognitive operations. Locally emerging oscillations and associated synchronous discharge of neurons can be temporally coordinated by slower rhythms so that information flows to just the right processing unit at just the right time. Because the numerous cortical rhythms have non-integer relationships, they cannot entrain each other for extended periods (Figure). Instead, they produce interference patterns that often take the appearance of 'complex noise' in the electro- and magnetoencephalograms; a state exactly half way between the predictable behavior of single oscillators and the unpredictability of chaos (Buzsáki, 2006).

Generation of simultaneously acting multiple oscillators requires special architectures involving dense local connectivity coupled with sparse interregional connections. Neocortical architecture and the multiple time scales generated by its rhythms allow the results of local computation to be distributed throughout the entire cerebral cortex; conversely, activity in local networks is under the constant supervision of computation in other parts, a process usually referred to as distributed or global processing.

Against this background, it should come as no surprise that any corruption or deterioration of the numerous constituents responsible for maintaining the complex system of brain oscillations can contribute to psychiatric symptoms. Recently, several laboratories have reported various quantitative alterations in gamma frequency oscillations in schizophrenia (Uhlhaas and Singer, 2006). Because this fast rhythm is largely under the control of perisomatic inhibitory basket cells, its impairment can be a consequence of previously reported decreases of the parvalbumin class of interneurons in schizophrenics. All major tranquilizers have a profound potentiating effect on slow thalamocortical oscillations. Furthermore, cannabinoid receptor activation, which decreases the release of both glutamate and GABA, interferes with the formation of transient cell assemblies, reflected by decreased power of several rhythms, although the firing rates of cortical cells remain unaltered.

Finally, virtually every psychiatric disease is associated with alteration of sleep rhythms, and it remains to be learned whether alterations of sleep patterns is a consequence or perhaps a primary deficit of the disease. Because the constellations of oscillations are custom tailored for individual brains, they constitute a rich source for phenotype characterization and provide a quantitative means for monitoring progression and alleviation of the disease. A great challenge left for systems neuroscience is to understand how brain rhythms contribute to cognitive operations of the cerebral cortex and whether drug- or other treatment-induced restoration of oscillations is causal to the healing process.

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## Stress-induced formation of new synapses in the amygdala

Why are memories of emotional events often very powerful and persistent? Why do war veterans or victims of severe stress continue to have vivid flashbacks of traumatic events from their past, while their cognitive abilities diminish? Stress disorders bring these questions into sharp focus,

because chronic stress has contrasting effects on different types of memories. Stress impairs memories of facts and events, which depend on synaptic plasticity in the hippocampus. In contrast, stress greatly amplifies emotional memories, particularly aversive memories of fearful and stressful events, which are processed by the amygdala. But little is known about the synaptic basis for this contrast.

We have studied the impact of stressful experiences on cells and synapses in the amygdala, by using a combination of behavioral, neuroanatomical, and electrophysiological techniques. Using this strategy, we have identified novel neural correlates of stress-induced plasticity in the amygdala, which are strikingly different from those observed in the hippocampus. Chronic immobilization stress for 10 days (2 h/day) causes dendritic growth in principal neurons of the lateral amygdala in rats. This stress-induced dendritic hypertrophy in the amygdala is in contrast to earlier reports of hippocampal atrophy following chronic stress (Vyas *et al*, 2002). Chronic stress also increases spine-density on these elongated dendrites in the amygdala (Mitra *et al*, 2005). Furthermore, using whole-cell recordings in amygdalar slices, we find that chronic stress, in addition to reducing spontaneous and evoked GABAergic inhibitory currents, amplifies excitatory post-synaptic currents mediated by glutamatergic NMDA receptors in the lateral amygdala. More detailed electrophysiological analysis indicates that the stress-induced amplification of NMDA currents is mediated largely by synapses on newly formed spines that only contain NMDA receptors. These data suggest that exposure to chronic stress forms so-called 'silent' or NMDA-only synapses in the amygdala, which in turn could enhance their capacity for further plasticity. This prediction was confirmed in two ways. First, we find that stress enhances NMDA-dependent long-term potentiation (LTP), a synaptic mechanism for learning and memory. Second, in an auditory fear conditioning paradigm, the same pairing of tone with weak footshock that has relatively little impact on unstressed animals causes abnormally high levels of fear in previously stressed animals. Thus, prolonged stress appears to leave its mark in the amygdala by forming new synapses with greater capacity for subsequent potentiation, thereby creating an ideal synaptic substrate for emotional symptoms observed in stress-related psychiatric disorders (McEwen and Chattarji, 2007).

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## Orexins rocketing to attention

Rarely has the discovery of a new neurotransmitter been met with the excitement seen after the identification of the orexins/hypocretins a decade ago. In 1997, De Lecea and co-workers (de Lecea *et al*, 1998) used a subtraction hybridization approach to isolate a gene expressed only in the hypothalamus; they arrived at the name hypocretin because of the hypothalamic localization of the peptide and its structural similarity to the incretin peptides. Contemporaneously, Yanagisawa and co-workers (Sakurai *et al*, 1998), studying orphan G protein-coupled receptors, isolated a peptide that elicited feeding in sated mice, naming the peptide orexin. For no other reason than that the word orexin is shorter, we will refer to orexin hereafter.

The discovery of a peptide that appears to be intimately involved in metabolism and body weight regulation has been met considerable interest. However, the finding that degeneration of orexin neurons is the cause of narcolepsy (Nishino *et al*, 2000), a disorder marked by excessive daytime sleepiness and attention deficits, changed interest to frenzy, resulting in over a thousand peer-reviewed papers being published since orexin was discovered 10 years ago. These papers have described the contributions of orexin to a dizzying array of functions, ranging from sleep and feeding to abuse and schizophrenia (Sakurai, 2007; Deutch and Bubser, 2007).

How can the small number of orexin cells, estimated at about 70,000 in humans and only 3,000 in rats, account for such a wide spectrum of involvement in health and disease? First, hypothalamic orexin neurons send axonal projections that reach almost the entire neuraxis, and can thereby influence multiple functions. Second, there is a broad distribution of the two orexin receptors, OX1R and OX2R, that are targeted by orexins A and B, the two mature peptides derived from processing of the preproorexin precursor.

However, given the extensive orexin innervation of the brain and a correspondingly extensive network of orexin receptors, how can there be any specificity in orexin's actions? There are probably two answers. First, not all orexin neurons are the same: subsets of orexin neurons project to different areas of the brain and receive different inputs that regulate their activity. Second, there may be some commonality of function that underlies, in part, the involvement of orexin in so many disparate functions. For example, orexin appears to promote attention, which in turn is a critical aspect of integrated activity in many domains, from food seeking and foraging to cognition.

A number of orexin antagonists have been developed, including those

that selectively target one or the other of the two orexin receptors and one that blocks both sites. In contrast, there are no orexin agonists that freely enter the brain. Several drugs appear to activate orexin neurons indirectly, such as amphetamine and modafinil. The development of specific agonists at orexin receptors may represent novel therapeutic approaches to a variety of disorders, including those of sleep and cognition.

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## Whole-genome association for complex psychiatric disorders

Whole-genome association (WGA) is a powerful approach for the study of the genetic basis of common psychiatric disorders, as illustrated by several early WGA findings, including The Wellcome Trust Study (2007). The above-mentioned study was illustrative of the general potential of WGA for complex diseases, identifying loci for seven common diseases (including one genome-wide significant locus for bipolar disorder) by comparing 2000 cases with each disease to a common set of 3000 controls. The WGA technology, on the other hand, is an opportunity for hypothesis-free testing on a massive scale, leading to continued concerns for problems of non-replication, and inconsistency that have marked previous searches for candidate gene markers in complex diseases (Ionaddis, 2007).

The Wellcome Trust Case Control Consortium used a conservative statistical standard and evaluated only alleles, either directly genotyped or imputed, with a frequency of $>5\%$. Like other WGA studies, it also identified other regions that could be validated or replicated in other ways. The results from this study and other recent WGA studies are encouraging, but sobering. For the seven diseases in the Wellcome Trust Case Control Consortium, a total of 24 genome-wide significant signals were found. However, only one was found for bipolar disorder, at Chr 16p12 where interesting candidate genes are located. This region, however, is not the site of several regions previously implicated in family linkage studies. Furthermore, and just 'off the presses', WGA results for bipolar disorder presented at the World Congress of Psychiatric Genetics did not replicate the 16p12 locus, while implicating new, novel, regions.

The risk genotypes of the 16p12 locus exerted an effect on odds ratio for bipolar disorder of only about 2.1 (consistent with the common allele/moderate effect model) and, even together with other promising loci that fell below the genome-wide statistical threshold, accounted for a relatively small fraction of the genetic risk. Indeed, more than two-thirds of the 24 loci identified for the common diseases led to odds ratios of $<2$. Even larger sample sizes will be required to detect alleles of smaller effect. Furthermore, the important roles of genetic heterogeneity (the scenario of many rarer alleles), and copy number variations are increasingly being recognized.

In this regard, recent whole-genome analysis has revealed both copy number variations and single-nucleotide polymorphisms (SNPs) that can alter patterns of mRNA expression, but the SNP variation currently being captured in WGA studies does not track most of the effects of the copy number variants (Stranger *et al*, 2007). Family-based linkage (meiotic linkage, locus-based linkage) can be readily detected in the face of within-gene allelic heterogeneity, leading to mismatch of results with WGA, which is allele specific. The genetic origins of psychiatric disease remain largely unknown and the ultimate validation of the WGA findings will lie at the level of functional loci, a level where there are even fewer successes. WGA is already the source of new clues to the complex origins of psychiatric disease, but multiple and complementary approaches will be required to piece together the mosaic.

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## Imaging genetics offers new predictive markers of individual differences in behavior and risk for psychiatric diseases

Individual differences in trait affect, personality, and temperament are important predictors of vulnerability

to neuropsychiatric disorders, including depression, anxiety, and addiction. Accordingly, identifying the biological mechanisms that give rise to trait individual differences affords unique opportunity to develop both predictive markers of disease liability and identify novel targets for individualized treatment.

In the past 5 years, human neuroimaging studies, especially those employing BOLD fMRI, have begun to reveal the neural substrates of interindividual variability in these and related constructs. Moreover, recent studies have established that BOLD fMRI measures represent temporally stable and reliable indices of brain function (Manuck *et al*, 2007; Johnstone *et al*, 2005). Thus, much like their behavioral counterparts, patterns of brain activation represent enduring, trait-like phenomenon, which in and of themselves may serve as important markers of liability and pathophysiology. As neuroimaging studies continue to illustrate the predictive relationship between regional brain activation and trait-like behaviors (eg, increased amygdala reactivity predicts core features of anxious temperament), an important next step is to systematically identify the underlying mechanisms driving variability in brain circuit function. In this regard, recent neuroimaging studies employing pharmacological challenge paradigms, principally targeting monoamine neurotransmission, have revealed that even subtle alterations in dopaminergic, noradrenergic, and serotonergic signaling can have profound impact on the functional response of brain circuitries supporting affect, personality, and temperament. Similarly, multimodal neuroimaging approaches have provided evidence for directionally specific relationships between key components of monoaminergic signaling cascades, assessed with radiotracer PET, and brain function, assessed with BOLD fMRI (Fisher *et al*, 2006). Collectively, pharmacological challenge neuroimaging and multimodal PET/fMRI are revealing how variability in behaviorally relevant brain activation emerges as a function of underlying variability in key brain neurotransmission systems (eg, increased serotonin signaling predicting increased amygdala reactivity).

The next logical step is to identify the sources of interindividual variability in these key neurochemical signaling mechanisms. In the modern era of human molecular genetics, this step is firmly planted in the direction of identifying the relationships between common variation in the genes encoding components of these signaling cascades, their protein products, and subsequently, brain circuit function. As sequence variation across individuals represents the ultimate wellspring of variability in emergent neurobiological and related behavioral processes, understanding the relationships within genes, brain, and behavior is critical for establishing the etiology and pathophysiology of psychiatric disease. The emerging field of imaging genetics seeks to establish a principled framework for the integration of modern molecular genetics and neuroimaging technologies towards the ultimate goal of identifying truly predictive makers of disease vulnerability (Hariri and Weinberger, 2003). The vast potential of such an integrated approach has been highlighted in recent studies whose collective results demonstrate that common sequence variation in the human serotonin transporter gene is associated with downstream alterations in serotonin signaling cascades that result in relatively increased serotonin signaling and, eventually, increased amygdala reactivity to environmental threat. This genetically driven variability in serotonin neurotransmission and threat-related amygdala reactivity likely represents a key mechanism of increased temperamental anxiety and risk for depression, especially in the context of environmental adversity. With increased utilization of such imaging genetics strategies and their continued expansion to include pharmacological and multimodal neuroimaging techniques, many more behaviorally and clinically relevant neurbiological pathways and predictive markers will be illuminated in forthcoming years.

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## Genes and modeling of schizophrenia: the curse of plentitude?

Rapidly growing knowledge about the neurobiology and genetics of schizophrenia stimulated new interest in animal models, which are used to dissect the molecular mechanisms of pathophysiological abnormalities in schizophrenia and create more effective therapies. The concepts about how to approach animal modeling of this complex, multifactorial (ie, involving multiple genes and a variety of epigenetic causes) neuropsychiatric disorder have been evolving over years and reflect the changing ideas about the etiology and the mechanism of the illness (Chen *et al*, 2006). These past approaches included pharmacological manipulations of dopamine and glutamate systems, thought to be in the center of the neurotransmitter imbalance in schizophrenia and the main culprits of its psychotic

symptoms and cognitive impairments. Subsequent concepts focused on disruptions of early brain development to address the evidence that the disorder has a neurodevelopmental origin; the onset of schizophrenia is typically in adolescence or early adulthood, and early childhood is not normal in many cases. Manipulations of psychosocial environment and induction of stressful conditions have also been considered as model targets due to the evidence that stress is involved in precipitating the illness.

Most recently, however, a new wave of models based on the breakthroughs in the discovery of human schizophrenia susceptibility genes has yielded the most fascinating results. Although they brought us a bit closer to understanding the functions of some 'faulty' genes, they have also raised more questions about the functions of the putative susceptibility genes and their role in the human disorder. Many of the schizophrenia candidate genes (eg, COMT, GRM3, PPP3CC (calcineurin), DARPP32) have been associated with cognitive dysfunction, a symptom relatively resistant to current antipsychotic treatments and viewed as a core symptom of schizophrenia. The genetic animal models with mutations in the genes involved in brain development (eg, DISC1, NRG1, DTNBP1) have provided insights into molecular mechanisms of abnormal neurodevelopment in schizophrenia. In particular, several recent studies on disruptions of the DISC1 gene in mice not only illustrate great potential of the new genetic approaches but also signal the vast complexity of the problem. An initial rationale for studying the effects of mutations in DISC1 came from the discovery of the chromosomal translocation, resulting in a breakpoint in the DISC1 gene that co-segregated with major mental illness in a Scottish family (reviewed by Porteous *et al*, 2006). These clinical findings were followed by a number of association studies, which reported that numerous SNPs across the gene were associated with schizophrenia and mood disorders and a variety of intermediate phenotypes, suggesting that other problems in the DISC1 gene may exist in other subjects/populations.

Animal models constructed to mimic partial loss of DISC1 function suggested that DISC1 is necessary to support development of the cerebral cortex, as its loss resulted in impaired neurite outgrowth and the spectrum of behavioral abnormalities characteristic of major mental disorders (eg, Hikida *et al*, 2007). Unexpectedly, however, another DISC1-knockdown model, achieved by RNA interference in single cells of the dentate gyrus, demonstrates that DISC1 may also function as a brake on neuronal development, and that its loss could lead to the opposite effects—dendritic overgrowth and accelerated synapse formation, and maturation of newly generated neurons (Duan *et al*, 2007). Other emerging studies continue to reveal the highly complex nature of the DISC1 gene with multiple isoforms exhibiting different functions, perhaps depending on localization, timing, and interactions with a multitude of other genes' products, some of which confer susceptibility to mental illness in their own right. Similar molecular complexity has also emerged in other susceptibility genes for schizophrenia, including GRM3 (Sartorius *et al*, 2006), NRG1 (Tan *et al*, 2007), and COMT (Tunbridge *et al*, 2007). With the growing knowledge of transcript complexity, it becomes increasingly clear that subtle disturbances of isoform(s) of susceptibility gene products and intricate interactions between the susceptibility genes may account for the etiology of neuropsychiatric disorders. Animal research will play a critical role in disentangling the web of genetic pathways.

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## Is it time for cannabinoid antagonists?

One of the recent remarkable events in therapeutic neuroscience has been the approval of rimonabant, the first cannabinoid receptor Type 1 antagonist used by the European Union (EU) for treatment of obesity, hyperlipidemia, and glucose intolerance. This achievement was preceded by decades of intense scientific work on the cannabinoid field. This has led to the identification and cloning of two specific receptor types, the characterization of endogenous ligands, and the synthesis of numerous antagonists and agonists. Patents are now held on over 100 cannabinoid CB-1 (CB-1) antagonists. Preclinical studies indicate that CB-1 receptor antagonists have major modulating effects on natural reinforcers, such as food and addictive drugs such as cocaine, opiates, nicotine, and alcohol. Peripheral

effects of CB-1 antagonists include increased thermogenesis, increased peripheral lipogenesis, and improvement in glycemic utilization. The treatment potential for addictive disorders, obesity, and the metabolic syndrome are evident (Gelfand and Cannon, 2006).

Acute administration of CB-1 antagonists is effective in reducing drug-seeking behavior in many rodent models (see Maldonado *et al*, 2006). For example, it suppresses self-administration of heroin in rodents, development of conditioned place preference in mice and rats, heroin reinstatement by a small priming injection of heroin, and aquisition of alcohol-drinking behavior and the alcohol-deprivation effect in two lines of alcohol-preferring rodents. Self-administration of exogenous cannabinoids is also suppressed by acute administration of CB-1 antagonists. CB-1 antagonists do not acutely suppress self-administration of cocaine, but appear to attenuate reinstatement of cocaine priming. CB-1 receptors are located on afferent pathways to the ventral tegmental area on GABAergic and glutaminergic neurons. It is postulated that CB-1 antagonists reduce inhibitory cannabinoid tone, specifically on GABAergic interneurons in the ventral tegmental area, leading to increased GABA inhibition of dopamine neurons (Carai *et al*, 2005).

Despite the enormous therapeutic potential of CB-1 antagonists for the treatment of obesity, metabolic, cardiovascular, and addictive disorders, their treatment future in US remains uncertain. In 2006, EU approved rimonabant for the treatment of obesity and selected aspects of the metabolic syndrome; it is now approved in over forty countries worldwide. In June 2007, the Food and Drug Association failed to give approval of rimonabant for the treatment of obesity in US, citing concerns over central nervous system side effects, particularly depression, anxiety, and increased rates of suicide. Several other CB-1 antagonists are now well into Phase III trial in US for the treatment of obesity and the metabolic syndrome. If safety concerns for CB-1 antagonists are resolved during post-marketing surveillance in EU, it is very likely that efficacy and safety clinical trials will be initiated for several addictive disorders. The off-label use of rimonabant for the clinical treatment of addiction is occurring outside US (FDA Advisory Committee, 2007).

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**DISCLOSURE/CONFLICT OF INTEREST**

I, Robert Malcolm, declare that, except for income received from my primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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## Purposeful design and animal models lead to the discovery of a novel class of antipsychotic drugs

Multidisciplinary lines of evidence have led to the emergence of the glutamate synapse as a key component of the pathophysiology of schizophrenia. Based on these findings, several leading theories have been introduced into the field and have been the basis for identification of novel therapeutic targets for treatment of schizophrenia. A recent proof-of-concept clinical trial paper shows promising results with one of these compounds (Patil *et al*, 2007). This double-blind placebo-controlled study showed that after 4 weeks of treatment, an agonist for the metabotropic glutamate 2/3 receptor (mGlu2/3R) is as effective as olanzapine in ameliorating positive and negative symptoms of schizophrenia. At both practical and conceptual levels, these findings have the potential of revolutionizing the field.

At a practical level, this represents one of the first times where purposeful design and laboratory-based basic and clinical research has led to the discovery of a truly novel target with clinical efficacy for a major psychiatric disorder. It is a fantastic demonstration that translational research works in psychiatry. It also underscores the importance of mutual interactions between pharmaceutical companies and (mostly NIH and NSF supported) academic research. The first major step was the discovery, by academic scientists, of metabotropic glutamate receptors and rapid characterization of their molecular and functional characteristics. Then came Lilly's foresight, led by Darryl Schoppe and talented chemists like Jon Monn to develop selective ligands for these receptors. The crucial next step was Lilly's willingness to share these compounds, in particular mGluR2/3 agonists, with academicians like myself. This led to the testing of these compounds in hypothesis-driven experiments related to several brain disorders. We used this compound because of its capacity to inhibit the release of glutamate to test our working hypothesis that a state of NMDA deficiency in schizophrenia causes excess release of glutamate in the prefrontal cortex. We postulated that reducing excess glutamate neurotransmission would have antipsychotic efficacy.

In our hands, this compound reversed key neurochemical, behavioral, and electrophysiological effects of NMDA deficiency in the prefrontal cortex (Moghaddam and Adams, 1998). Work by Aghajanian and Marek also showed the effectiveness of mGlu2/3R activation on reversing the hyperglutamatergic effects of LSD

on prefrontal cortex cells (Marek *et al*, 2000). The work was then translated to human laboratory experiments demonstrating that mGlu2/3R agonists reduced key deficits in a human model of NMDA deficiency in healthy volunteers (Krystal *et al*, 2005). Thus, although mGlu2/3R agonists did not work in traditional animal models of schizophrenia such as prepulse inhibition, other mechanistically driven work provided critical support for the therapeutic efficacy of this class of compounds in schizophrenia. The final step was, of course, Lilly's nearly decade long commitment to carry out the proof-of-concept clinical trial.

Conceptually, the results of this clinical trial will compel the field to reevaluate its three leading theories on schizophrenia: 'the glutamate hypothesis;' 'the hypofrontality model;' and, of course, 'the dopamine hypothesis.' This will be a welcome change. Although at the outset this work has been hailed as supporting the glutamate hypothesis, that hypothesis is generally perceived as a state of glutamate deficiency. Lilly's mGlu2/3R agonists reduce glutamate release. These compounds also reduce spontaneous and activated neuronal firing in the prefrontal cortex of awake animals (Homayoun *et al*, 2004), suggesting that they would exacerbate a state of cortical 'hypoactivity'. The implications for the dopamine hypothesis are obvious. The all too common statement 'all known antipsychotic drugs block dopamine receptors' no longer holds true.

The trial, of course, must be replicated. It is possible that this particular class of compounds will not be useful for long-term treatment. Agonists in general are not good options for sustained treatment; therefore, newer compounds that allosterically modulate mGlu2/3R may be needed. Meanwhile, there is great optimism that this proof-of-concept study is eliciting a much needed paradigm shift in the field.

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## Synaptic dysfunction in Rett Syndrome, an autism spectrum disorder

Rett syndrome (RTT) is a pervasive developmental disorder that accounts for one of the leading causes of autistic behavior and mental retardation in females. Recent work has identified key functional abnormalities associated with RTT. It has been known for several years that RTT is an X-linked dominant disorder that in the vast majority of cases (>96%) results from mutations in the coding region of the Methyl-CpG-binding protein 2 (MeCP2) gene, leading to its loss of function (Amir *et al*, 1999). Normally, MeCP2 binds to methylated cytosines in the DNA of target gene promoters and acts as a transcriptional repressor by silencing their transcription; therefore, loss of MeCP2 is expected to result in inappropriate upregulation of gene expression. However, microarray analyses from brain tissue of MeCP2-null knockout mice have shown only subtle changes in gene expression and failed to identify genes that are relevant to the pathology of RTT. Mutations in the MeCP2 gene have been identified in other patient populations, including Angelman Syndrome, autism, and mental retardation syndromes. Duplication of the MeCP2 gene has also been identified in some male patients with mental retardation and progressive neurological symptoms. These findings suggest that alterations in MeCP2 expression can contribute to disease progression with strong neurological phenotypes, and that regulation of MeCP2 expression must be tightly controlled under normal circumstances.

Over the past couple of years, research has focused on whether loss of MeCP2 expression impacts functional alterations in synaptic transmission that may underlie the disease phenotype. Recently, researchers have discovered a number of defects in synaptic function in different mouse models of RTT. It appears that the loss of MeCP2 function can lead to changes in spontaneous synaptic transmission as well as in short- and long-term synaptic plasticity. Two studies, one using a MeCP2-null mouse (Asaka *et al*, 2006) and another using a mouse expressing a truncated form of MeCP2 (Moretti *et al*, 2006), found deficits in both long-term potentiation and long-term depression in hippocampal slices from these mice compared to littermate controls. Interestingly, whereas the first study saw these changes only in older, symptomatic mice (Asaka *et al*, 2006), the second found them also in younger, asymptomatic mice, suggesting the possibility that these synaptic deficits may be occurring before the manifestation of RTT-like behaviors (Moretti *et al*, 2006).

Additional defects in basal synaptic transmission were seen in these as well as two other studies. In cortical pyramidal neurons, spontaneous and miniature excitatory postsynaptic current (EPSC) properties were

reduced in MeCP2-knockout mice, whereas a small increase was seen in the overall synaptic charge of spontaneous inhibitory postsynaptic currents (IPSCs) (Dani *et al*, 2005). In dissociated hippocampal cultures, we found a significant decrease in the frequency of spontaneous mEPSCs in MeCP2-knockout versus control neurons, whereas no change was seen in mIPSC properties (Nelson *et al*, 2006). These changes in synaptic transmission, in particular, short-term plasticity, may be quite important in underlying mechanisms of neurological dysfunction observed in RTT patients. In addition, each of these studies investigating MeCP2 and synaptic transmission point to a possible imbalance between excitatory and inhibitory activity in the brains of MeCP2 mutant mice, perhaps towards less excitation and therefore more inhibition, but this needs to be explored further. It has been suggested that there may be an abnormal ratio of excitation/inhibition in the brains of autistic patients, and as RTT is considered an autism-spectrum disorder, it is not unreasonable to hypothesize that something similar may be occurring in the brains of RTT patients. Future work in this area promises to yield important information towards our understanding of RTT as well as related autism spectrum disorders.

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## Evidence that dopamine response to amphetamine sensitizes in humans

There is general agreement that repeated exposure to psychostimulant drugs leads to behavioral sensitization. In some preparations, behavioral sensitization is accompanied by long-lasting enhanced increases in extracellular levels of ventral striatal dopamine (DA) in forebrain. This finding has figured importantly in recent theories of addiction, proposing that sensitized DA overflow acts in concert with other alterations in the neurochemistry of ventral striatum (nucleus accumbens) to enhance the appetitive effects of drugs and promote their pursuit and self-administration (Robinson and Berridge, 1993; Vezina, 2004). However, experimental support for enhanced DA overflow stems from rodent studies, whereas findings obtained in nonhuman primates and addicted individuals have been equivocal. For example, PET studies in cocaine-addicted individuals have reported reduced rather than augmented drug responses in striatal regions (eg, Volkow *et al*, 1997). This has led to arguments that DA increases associated with drug sensitization as a mechanism for drug abuse and other forms of pathology is of limited value to the human condition. Recent evidence has emerged, however, demonstrating that drug sensitization does in fact occur in humans. In experiments using PET to assess endogenous DA displacement of [$^{11}$C]raclopride in humans, investigators at McGill University in Montreal have found that individual differences in drug-induced DA release correlate positively with the personality trait of novelty seeking and drug-induced wanting, and that acute DA depletion decreases both drug craving and work undertaken to obtain the drug. Significantly, individuals administered amphetamine 2 weeks and up to 1 year after being repeatedly exposed to the drug exhibit enhanced DA release in ventral striatum that also extends to dorsal striatal regions (Boileau *et al*, 2006). These results indicate that continued investigative effort needs to be directed not so much at determining whether or not sensitization can be demonstrated in different species, but rather at characterizing the nature of its impact on the generation of appetitive behavior and delineating when and under what circumstances it is produced. Experience with drug self-administration, for example, is not a sufficient condition for the development of sensitization either in rodents (Roberts *et al*, 2007) or in nonhuman primates (Bradberry, 2007). Those procedures that do reliably produce sensitization need to be characterized and their underlying neurobiology understood.

As proposed by Leyton (2007), the different findings obtained in human studies may reflect the effects of different drug exposure regimens and withdrawal periods in non-drug-abusing compared to drug-abusing human subjects. Notably, human addicts may require a long abstinence period before sensitization can be detected. In addition, drug-paired and drug-unpaired cues may differentially influence drug-induced DA responsivity in these two groups. The constellation of stimuli afforded by the PET testing environment will notably exert different effects in individuals who have received drug only in their presence, compared to others who have associated these cues with the absence of drug. The demonstration that drug exposure can sensitize DA responding in human subjects highlights the need to consider and evaluate these as well

as other possible factors capable of modulating the impact of sensitization on behavior.

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DISCLOSURE/CONFLICT OF INTEREST
This work was supported by a grant (DA09397) from the National Institutes of Health to PV. The author declares that, except for this grant and income received from his primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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## Modulating endogenous cannabinoids to treat pain and affective disorders

The endocannabinoids are a family of biologically active lipids that activate cannabinoid (CB) receptors, the G protein-coupled receptors targeted by $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) in marijuana. The term encompasses several derivatives of the polyunsaturated fatty acid arachidonic acid, including anandamide (arachidonoylethanolamide), and 2-arachidonoylglycerol (2-AG). The endocannabinoids are thought to operate primarily as paracrine mediators—substances that are generated on demand by neurons and other cells in response to physiological stimuli and act in the vicinity of their sites of synthesis (Piomelli, 2003).

In brain, the endocannabinoids may mediate localized signaling mechanisms through which neurons modify the strength of incoming synaptic inputs. For example, evidence indicates that 2-AG is generated in the hippocampus by activation of postsynaptic metabotropic glutamate mGlu5 receptors and travels backwards across the synapse to inhibit glutamate and GABA transmission, a process called 'retrograde signaling' (Hohmann *et al*, 2005). Other data suggest that local release of anandamide in the dorsal raphe nucleus, locus coeruleus, and periaqueductal gray matter regulates the activity of ascending and descending aminergic pathways to influence stress responses, pain, and affect (Hohmann *et al*, 2005; Gobbi *et al*, 2005).

The proposed role of the endocannabinoids in the control of pain and emotion has both theoretical and clinical interest and it could be exploited to develop novel analgesic, anxiolytic, and antidepressant drugs. However, the psychotropic properties and abuse liability of direct-acting CB receptor agonists such as $\Delta^9$-THC pose a major obstacle in the realization of this therapeutic potential. One possible way to circumvent such an obstacle might be to develop drugs that prevent the biological deactivation of the endocannabinoids and, by doing so, amplify their intrinsic effects in a site- and context-restricted manner.

Anandamide and 2-AG are rapidly eliminated through a two-step process, consisting of uptake into cells and enzymatic hydrolysis. The two endocannabinoids share what appears to be a functionally similar transport mechanism, but follow distinct routes of intracellular degradation. Inside cells, anandamide is metabolized by fatty acid amide hydrolase (FAAH), a membrane-bound serine hydrolase that is found in neuronal cell bodies throughout the cortex. 2-AG hydrolysis is catalyzed instead by monoacylglycerol lipase, a cytosolic serine hydrolase that is localized in presynaptic terminals. Agents that target these deactivating reactions might display a more selective pharmacological profile than direct CB agonists. For example, inhibitors of intracellular FAAH activity were shown to exhibit marked anxiolytic, antidepressant, and analgesic effects in rodents (Gobbi *et al*, 2005; Kathuria *et al*, 2003; Russo *et al*, 2007). These behavioral effects are accompanied by augmented brain levels of anandamide and are prevented by $CB_1$ cannabinoid receptor blockade, but are not associated with overt psychotropic or rewarding actions (Gobbi *et al*, 2005). FAAH inhibitors have recently entered clinical trials, and data regarding their efficacy in patients should become available in the next few years. Discovery efforts targeting other endocannabinoid-deactivating pathways are also underway.

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DISCLOSURE/CONFLICT OF INTEREST
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# Raising the bar in drug development for depression: antidepressant response in hours instead of weeks

All the currently available antidepressant medications exhibit a delayed onset of antidepressant response, and often take weeks to months to achieve their full effects; this commonly results in considerable morbidity, disruption to personal, professional, family, and social life, and high risk for suicidal behavior. Any antidepressant treatment that shifts the time frame of response from weeks to a few hours would undoubtedly revolutionize the care of the many millions who suffer from depression.

To date, a lack of understanding of the precise molecular underpinnings of currently effective antidepressants has hampered our ability to develop novel therapeutics that work more quickly than existing treatments. However, the demonstration that the NMDA antagonist ketamine induces a rapid antidepressant response within hours has led to exciting new research into cellular mechanisms that affect rapid antidepressant action.

Whereas most antidepressants exert their initial effects by increasing the intrasynaptic levels of serotonin and/or norepinephrine, the resolution of core depressive symptoms becomes manifest only after weeks or months of chronic administration, suggesting that alterations in downstream signaling cascades and, ultimately, synaptic plasticity, are responsible for their therapeutic effects (Zarate *et al*, 2006). Furthermore, accumulating evidence suggests that alterations in the regulation of glutamatergic neurotransmission contribute to the pathophysiology of depression, as well as the mechanism of existing antidepressants. This supporting evidence comes from (1) demonstration of glutamatergic abnormalities in patients with depression, (2) effects of existing antidepressant and mood-stabilizing medications on the glutamatergic system, (3) preclinical evidence suggesting that drugs targeting various components of glutamate neurotransmission possess antidepressant and anxiolytic properties, and (4) recent studies demonstrating the effectiveness of glutamate-modulating agents in the treatment of mood disorders.

Studies have demonstrated that a single subanesthetic dose of the NMDA antagonist ketamine, when given intravenously, induces a rapid (within hours) antidepressant effect (Berman *et al*, 2000; Zarate *et al*, 2006); furthermore, in the most recent study of treatment-resistant depression, the antidepressant effect of a single subanesthetic dose of ketamine was sustained for 1–2 weeks. In that study, the response rates obtained with ketamine were comparable to those that occur after 8 weeks of chronic treatment with current monoaminergic-based antidepressants (Zarate *et al*, 2006).

To our knowledge, there has never been a report of any other somatic or pharmacological intervention that consistently and reproducibly results in such a dramatically rapid and prolonged response—well beyond the half-life of the drug—with a single administration. We postulate that the rapid antidepressant response to ketamine is unlikely to result from major 'neural remodeling,' but rather occurs via alterations in 'here and now' synaptic changes. Recent studies show that chronic administration of antidepressants enhances synaptic/surface AMPA receptors. Notably, ketamine rapidly increases the release of glutamate (Moghaddam *et al*, 1997), a process probably mediated by NMDA autoreceptors, and/or by GABAergic interneurons. A series of studies were undertaken to test the hypothesis that the therapeutic effects of both monoaminergic antidepressants and ketamine may be mediated by increased AMPA to NMDA throughput in critical neuronal circuits. It was hypothesized that ketamine would do this directly—by increasing glutamate release and concurrently blocking postsynaptic NMDA receptors—whereas monoaminergic antidepressants would do this indirectly and gradually, by producing delayed effects on AMPA and NMDA subunit phosphorylation and trafficking. Indeed, recent biochemical and behavioral studies support such a contention (Maeng *et al*, 2007).

As the search for treatments in depression continues, it is crucial to change the way we understand and conduct drug development. As with other areas of medicine, our gradual understanding of the pathophysiology of depression and mechanism of action of antidepressants indicates that an antidepressant response that occurs within hours is now an obtainable goal. The work described above provides direct evidence that rapid response is possible. It is our belief that our current expectations regarding antidepressant treatments are too low; instead of developing treatments that take weeks to induce response, we should begin to develop drugs that instead resolve depression within hours.

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**DISCLOSURE/CONFLICT OF INTEREST**

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