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LTD deficit in $\alpha 7$ neuronal nicotinic receptor ($\alpha 7^*$) knockout mice is strain dependent

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Schizophrenia is a mental disorder characterized by insufficient filtering of external stimuli as well as other cognitive deficits. The prevalence of this disorder (~1%) and evidence of its heritability give reason to investigate the genetics and functional neuronal interactions which may contribute to schizophrenia. The alpha 7 neuronal nicotinic receptor is thought to play a role in the development or expression of schizophrenia. Postmortem brain tissue from schizophrenics has fewer alpha-bungarotoxin binding sites – indicative of alpha 7 nicotinic receptors – than age-matched control individuals. It has been proposed that schizophrenic patients smoke at high levels and with disproportionately high incidences in order to compensate for the lack of these receptors. Furthermore, schizophrenics exhibit abnormal auditory gating, an endophenotype of the disorder, and administration of nicotine i.v. normalizes gating to auditory stimuli. Since cognitive deficits are frequently observed in schizophrenics, we hypothesized that mice which lacked the alpha 7 nicotinic receptor might mimic some of schizophrenic phenotypes, including deficits in synaptic plasticity. Previously we investigated long-term potentiation (LTP) in hippocampal slices from mice which lacked the alpha 7 nicotinic receptor gene (knock outs, KO), and we compared those data with that from wild type (WT) slices. We found that high-frequency stimulation induced less LTP in KO mice than in WT mice, but this difference depended on the background genotype; the deficit was apparent in C3H mice, but not in mice with the C57 background genotype. We have now examined another form of synaptic plasticity – long-term depression (LTD) – from these animals. In order to observe this effect, we tested mice at 13–19 days of age. Low-frequency stimulation (LFS; 1 Hz, 15 min, 900 pulses) evoked ~30% LTD in WT mice of the C3H background for up to 1 h after LFS. Similarly to LTP data, C3H mice lacking the alpha 7 receptor exhibited a deficit in LTD (essentially no LTD); Het mice were intermediate, exhibiting a small degree of LTD. Preliminary data also indicated that in mice of the C57 background, there was only a slight, nonsignificant difference in the LTD observed for KO vs. WT hippocampal slices (LTD: WT \geq KO). Future experiments will determine whether the LTP and LTD deficit in C3H mice lacking the alpha 7 receptor is due to a developmental alteration or a dynamic, acute effect of the KO, which can be inhibited in WT mice by the appropriate nicotinic antagonist, or which can be overcome in KO slices by a nicotinic agonist.

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lynx1 balances neuronal activity through nicotinic acetylcholine receptor modulation

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Maintenance of the level and function of nicotinic acetylcholine receptors (nAChRs) during development and adulthood is essential for proper circuit function. The action of lynx1, a prototoxin with structural similarity to α -bungarotoxin, manifests itself at both circuit and network levels on nicotinic systems. Removal of the molecular brake provided by lynx1 can lead to nicotinic receptor hypersensitivity—larger direct nicotinic responses, slowed desensitization kinetics, and enhanced sensitivity to nicotine. As a consequence of nAChR hypersensitivity, lynx1 knockout (KO) mice display increased levels of Ca^{2+} in neurons, enhancements in synaptic efficacy, and improved learning and memory functions. Studies on such hyperactive nicotinic receptors can reveal cholinergic-dependent processes with increased clarity. For instance, adult lynx1 KO mice display heightened ocular dominance plasticity after the normal close of the critical period, suggesting that lynx1 acts as a molecular brake over the cholinergic system to suppress plasticity in the adult brain. nAChRs have been implicated in neuronal maturation and loss of synaptic lability, which appear to be correlated with the onset of lynx1 expression. $\alpha 4\beta 2$ and $\alpha 7$ nAChRs in particular are known to regulate the excitatory/inhibitory balance. Our hypothesis is that lynx1 controls excitatory/inhibitory balance by regulating the number and localization of nAChRs. This modulation may occur through intracellular mechanisms or via cell surface gating interactions. Using high-resolution live cell imaging of nAChRs labeled with fluorescent proteins we have monitored receptor movement and localization in the presence and absence of lynx1. Total internal reflection microscopy (TIRF) data indicate that lynx1 increases the plasma membrane to ER ratio of nAChRs. Mobile fluorescent puncta were analyzed by creating kymographic representations of time-lapse TIRF images. lynx1 caused a 96% increase in mobile puncta, indicating that lynx increases nAChR mobility. We are also employing immunohistochemical and western blot analyses to monitor the state of neuronal maturation. These data indicate that lynx1 influences nAChRs to maintain excitatory/inhibitory balance in neuronal circuits. Thus, these data indicate how a prototoxin may function, at an intracellular level, to alter the level and localization of nAChRs, with profound implications on cellular and circuit function.

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Altered spine configuration in CA1 hippocampal basilar dendritic spines in alpha7 nAChR knockout mice

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The alpha7 nicotinic acetylcholine receptor (nAChR) is known to be involved in cognition and memory functions. The mechanisms underlying the learning-related functions of the alpha7 nAChR are not well-understood. The receptor is widely expressed in the

brain, but densely localized in both pre- and postsynaptic sites on interneurons innervating hippocampal pyramidal cell dendrites. In an effort to understand possible structural changes that might be related to learning-related behavioral deficits known to occur in $\alpha 7$ knockouts, we quantified several measures of dendritic morphology in the CA1 region of the mouse hippocampus in wild-type and knockout $\alpha 7$ mice. Measurements were made in Golgi-stained material from formalin fixed brains in mice at 60 days of age. The most significant difference was a 63% increase in thin (L-type) dendritic spines on the CA1 basilar tree in knockout mice ($p = 0.0005$) and a small but significant decrease in N-type (Nubby) basilar dendritic spines (-15% , $p = 0.02$). There was no difference in the total number of spines per neuron and per brain between wild-type and knockout animals nor was there a difference in M-type and D-type spines in the basilar dendrites. In the apical CA1 dendritic tree, there was a significant increase number of total spines in knockouts ($+11\%$, $p = 0.01$). In the Parietal Cortex, layers II–III, there was a trend toward decreased dendritic branching between in knockout mice. No differences were found in measurements of dendritic branching of the granule cells of the dentate gyrus. In sum, the data suggests that the $\alpha 7$ nAChR knockout genotype results in an alteration in brain circuitry in cortical and hippocampal regions which could disrupt normal cognitive function.

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Mice lacking the $\beta 4$ subunit of the nicotinic acetylcholine receptor show memory deficits, altered anxiety- and depression-like behavior, and diminished nicotine-induced analgesia

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Nicotine binds to nicotinic acetylcholine receptors (nAChRs) throughout the brain and elicits a range of behavioral responses related to cognition, anxiety, depression and analgesia. However, the role of specific nAChRs subtypes in regulating behavior in the absence of nicotine is still not clear. We investigated the role of $\beta 4$ -containing nAChRs in behavioral tests assessing cognitive function, affective behaviors, and nociception using wildtype ($\beta 4^{+/+}$) and knockout ($\beta 4^{-/-}$) mice for the nAChR $\beta 4$ subunit. Mice were tested in a battery of cognitive tasks including the Y-maze (spontaneous alternations), the novel object and novel location recognition tasks, the Barnes maze, and the contextual and cued fear conditioning tasks. Anxiety- and depression-like behaviors were evaluated in the light-dark box, forced swim, tail suspension and marble burying tests. The analgesic effect of nicotine was investigated using the tail immersion and hot plate tests. There were no significant learning and memory deficits in $\beta 4^{-/-}$ mice compared to $\beta 4^{+/+}$ mice during acquisition of the Barnes maze, contextual fear conditioning, Y-maze, and the novel object recognition tasks. In the Barnes maze memory retention test, male $\beta 4^{-/-}$ mice showed reduced use of the spatial search strategy, indicating small spatial memory deficits compared to $\beta 4^{+/+}$ mice. In the cue-induced fear conditioning memory retention test, both male and female $\beta 4^{-/-}$ mice exhibited reduced freezing time compared to $\beta 4^{+/+}$ mice, reflecting decreased memory retention, while during task acquisition memory deficits were observed only in male $\beta 4^{-/-}$ mice. Small memory deficits were detected in a subset of $\beta 4^{-/-}$ male mice compared to the $\beta 4^{+/+}$ male mice in the novel location, but not the novel

object, recognition task. Compared to $\beta 4^{+/+}$ mice, $\beta 4^{-/-}$ mice exhibited decreased anxiety-like behavior in the light-dark box; while depression-like behavior in $\beta 4^{-/-}$ mice was decreased in the tail suspension test (females only) and increased in the forced swim test (males only) compared to $\beta 4^{+/+}$ mice. In nociception tests, male and female $\beta 4^{-/-}$ mice did not differ from their $\beta 4^{+/+}$ counterparts in basal nociception, but were less sensitive to the antinociceptive effect of nicotine in two tests of acute thermal pain, indicating that $\beta 4$ -containing nAChRs are involved in the modulation of nicotine-induced analgesia. In conclusion, the results indicate that (a) lack of the $\beta 4$ -containing nAChRs resulted in deficits in hippocampus- and amygdala-dependent short- and long-term memory functions; (b) $\beta 4$ -containing nAChRs are involved in anxiety- and depression-like behaviors, and (c) contribute to the analgesic effects of nicotine.

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Using zebrafish to fill the gap between *in vitro* and rodent models for nicotinic drug development

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Zebrafish can provide a valuable animal model to screen potential cognitive enhancing and anxiolytic nicotinic drugs. Zebrafish are economical and can provide a relatively quick indication of possible functional efficacy. With a complex nervous system and elaborate behavioral repertoire, zebrafish can provide a good intermediate model between *in vitro* receptor and cell-based assays and classic mammalian models for drug screening. In addition, the variety of molecular tools available in zebrafish makes them outstanding models for helping to determine the neuromolecular mechanisms for nicotinic drugs. In a series of studies, our lab has developed tests of cognitive function and stress response, which are sensitive to drug effects in a similar fashion as rodent models and humans for cognitive enhancement and alleviating stress response. We have determined the effects of nicotine, the prototypic nicotinic agonist, and nicotinic antagonists on cognitive function, exploratory behavior and stress response in a series of behavioral tests we have developed to reliably index these behavioral functions. The overall hypothesis of this line of investigation was that nicotine would have similar behavioral effects in zebrafish as in mammals when analogous tests of behavioral function are used. In particular, the 3-chamber task for learning and memory was shown to be sensitive to the cognitive enhancing effects of nicotine and has been useful in helping to determine neural mechanisms critical for nicotinic-induced cognitive enhancement. The novel tank diving test was shown to be a valid and efficient test of stress response. It is sensitive to the reduction of stress-related behaviors of the anxiolytic drugs diazepam and buspirone. Nicotine also causes stress-alleviating effects which can be interpreted as anxiolytic effects. As with mammalian species, nicotine significantly improves learning and memory at low to moderate doses with an inverted J-shaped dose-effect function. The nicotine-induced learning improvement in zebrafish is reversed with the nicotinic antagonist mecamylamine and is accompanied by increased brain dopamine levels, an effect which is also reversed with mecamylamine. Also as in mammals nicotine has anxiolytic effects in zebrafish. Nicotine significantly reduces bottom dwelling in the novel tank diving task. This effect is reversed by either $\alpha 7$ or $\alpha 4\beta 2$ nicotinic antagonist co-administration. In many respects nicotine