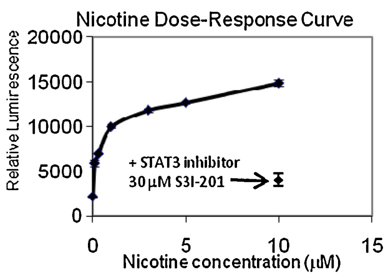
cells transfected with the reporter plasmid but lacking nicotinic receptors. In contrast, co-transfection with α7 in GH4-C1 cells allows dose-dependent nicotine-driven Stat3 signaling that is blocked by 30 μM Jak inhibitor AG-490 or 30 μM Stat3 inhibitor S3I-201. In order to see nicotine-driven α7-dependent Stat3 signaling in SH-EP1 cells, transfection of α7 with reporter plasmid requires co-transfection with the chaperone Ric3, which allows α7 receptors to traffic to the cell surface. We are investigating the effect of various agonists and antagonists on the effects of α7-mediated Stat3 signaling measured using this novel reporter plasmid. Future experiments will correlate nicotine-driven Stat3 signaling measured by secreted luciferase activity in cells expressing various nicotinic receptor subtypes with phosphorylated Stat3 levels measured by Western blotting.



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2.1

Section 2. In vivo pharmacology and clinical studies

Developmental nicotine exposure and the α5 nicotinic acetylcholine receptor

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Maternal smoking during pregnancy can expose the developing fetus to high concentrations of nicotine and has been linked with deficits in attention later in life. Layer VI pyramidal neurons of the medial prefrontal cortex (mPFC) may be a target for the teratogenic effects of nicotine because they are directly excited by nicotinic acetylcholine receptor (nAChR) stimulation during development, are a major source of feedback projections from the mPFC to the thalamus, and are believed to play an important role in attention. We sought to test this hypothesis by exposing mice to either nicotine tartrate (200 μg/mL calculated as nicotine free base) or tartaric acid control via maternal drinking water throughout gestation and up to weaning on postnatal day 21. Since the nAChR α5 subunit plays a critical role in the normal nicotinic response in these neurons, we tested its contribution to the developmental effects of nicotine by performing this study in both wild type (WT) and α5 subunit knockout (α5−/−) mice. We found a striking interaction between developmental nicotine exposure and α5 genotype during the third week of postnatal life, where the ability of both acetylcholine (in the presence of atropine to block muscarinic receptors) and nicotine to stimulate layer VI neurons was increased by developmental nicotine exposure only in α5−/− mice. In WT mice, by contrast, the α5 subunit appears to protect the nicotinic response in layer VI neurons from being changed by exposure to nicotine in development. The interaction between developmental nicotine exposure and α5 genotype occurred in the absence of changes to neuronal membrane properties. Since nicotinic stimulation can influence neuronal growth and maturation, ongoing experiments are investigating the effects of developmental nicotine exposure on mPFC layer VI neuron morphology. Moreover, since developmental nicotine-induced attention deficits can persist beyond developmental periods, we are also testing the effects of developmental nicotine exposure on mPFC layer VI neuron function and morphology in fully mature, adult mice.

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2.2

α6* nAChR expression and function in brain areas influencing DA transmission probed with α6-GFP transgenic mice

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Midbrain dopamine neurons serve critical functions in mediating arousal, motivation, motor control, and reward learning. The nicotinic cholinergic system, acting via β2* and α7* nAChRs, is an important regulator of DA transmission. Nicotinic ACh receptors containing the α6 subunit are expressed in a few select brain areas, including midbrain DA neurons, noradrenergic neurons of the locus coeruleus, and glutamatergic retinal ganglion cells. To better understand the regional and subcellular expression pattern of α6-containing nAChRs, we created and studied transgenic mice expressing a variant α6 subunit with GFP fused in-frame in the M3-M4 intracellular loop. α6-GFP receptors functioned normally *in vitro* in cultured cells, as well as *in vivo* in synaptosomal DA release experiments. In α6-GFP transgenic mice, α6 nAChR expression in the brain largely matched previous studies using radiolabeled α-conotoxin MII or mRNA *in situ* hybridization. Surprisingly, we also found α6 subunit expression in selected neuronal cell bodies in medial habenula, interpeduncular nucleus, and superior colliculus. MHb neurons expressing α6 subunits were located in the medial aspect of the MHb adjacent to the ventricle, which were completely distinct from α4-subunit containing MHb neurons located on the lateral aspect of the MHb. We also noted specific presynaptic and postsynaptic α6 expression in the ventral IPN. In the visual system, α6 subunits were strongly expressed in most retinal ganglion cells, and were weakly expressed in some neurons in dLGN and visual cortex. In superior colliculus, part of the extended basal ganglia critical for relaying short-latency salience signals to midbrain DA neurons, we found strong α6 expression in retinal axons, along with postsynaptic α6 expression in a fraction of SC GABAergic interneurons. In patch clamp recordings from mice expressing hypersensitive α6 subunits, we recorded α6-dependent, presynaptic and/or postsynaptic nicotinic responses in SC neurons. Together, these electrophysiological results demonstrate that α6* nAChRs are uniquely situated to mediate cholinergic modulation of glutamate and GABA release in SC. More globally, our results from these studies support the emerging hypothesis that α6* nAChRs, via their expression in key salience centers such as MHb/IPN, superior colliculus, locus coeruleus, and midbrain DA cell bodies and synaptic terminals, may be important for cholinergic sensitization of arousal and reward circuits.

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2.3

Alpha2* nicotinic acetylcholine receptors as a therapeutic target for memory enhancement

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GABAergic interneurons have a central role in the control of synaptic plasticity and hippocampus-dependent learning, and many of these interneurons express nicotinic acetylcholine receptors (nAChRs). However, it is largely unknown how activation of nAChRs on different interneuron subtypes influences hippocampal circuit activity or the induction of long-term potentiation (LTP) and long-term depression (LTD), which are considered to be cellular substrates of learning and memory. The α2* nAChR, the most sparsely expressed nAChR subtype in the brain, is selectively found in a subset of GABAergic interneurons in the stratum oriens/alveus. We have investigated the consequences of α2* nAChR activation on circuit activity and the induction of LTP and LTD. We found that it causes an increase in the frequency of spontaneous inhibitory postsynaptic currents in pyramidal cells in a glutamate-receptor-independent and Na^+ channel-dependent manner. Furthermore, dual whole-cell recordings from α2* nAChR-containing interneurons and pyramidal cells showed that pairs are synaptically connected. These results suggest that activation of α2* nAChRs causes GABA release onto postsynaptic membrane domains, affecting hippocampal circuit operation. We have also found that α2* nAChR-containing interneurons are continuously excited in the presence of nicotine and that Ca^{2+} entry through α2* nAChRs promotes the induction of *N*-methyl-D-aspartate receptor (NMDAR)-independent LTP in these interneurons. In addition, we found pathway specific effects of α2* nAChR activation. Hippocampal CA1 pyramidal cells, which provide the major output of the hippocampus, receive two major sources of excitatory synaptic inputs from the entorhinal cortex, the Schaffer collateral (SC) path and the temporoammonic (TA) path. Optical recordings with a voltage-sensitive dye showed that activation of α2* nAChRs enhances excitatory neural activity along the SC path, whereas activation of α2* nAChRs increases hyperpolarization along the TA path. Accordingly, activation of α2* nAChRs promotes the induction of NMDAR-dependent LTP at the SC path, but suppresses LTP induction at the TA path. In contrast, activation of α2* nAChRs has no significant effect on LTD induction at the SC path and facilitates LTD induction at the TA path. Our work shows that the α2* nAChR subtype is an important component of hippocampal circuitry and potentially serves as a switch for gating information flow and synaptic plasticity by exciting a subset of GABAergic interneurons in the stratum oriens/alveus. It is potentially involved in nicotine-induced cognitive enhancement, and appears to be an attractive new target for improving memory.

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2.4

Plasticity of prefrontal attention circuitry: Upregulated muscarinic excitability in response to decreased nicotinic signaling following deletion of α5 or β2 subunits

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Acetylcholine in the medial prefrontal cortex is critical for attention, an effect mediated by the ionotropic nicotinic and the metabotropic muscarinic families of cholinergic receptors. Corticothalamic pyramidal neurons in layer VI of the medial prefrontal cortex express cholinergic receptors of both families and play an important role in attention through their feedback projections to the thalamus. Response to acetylcholine in medial prefrontal layer VI pyramidal neurons is primarily mediated by α4β2α5 nicotinic receptors. Mice lacking either the accessory α5 subunit [1] or the ligand-binding β2 subunit [2] show weaker attentional performance. However, the presence of muscarinic cholinergic receptors in these neurons raises the possibility of plasticity in their cholinergic response. Here, we investigate the combined effects of nicotinic and muscarinic cholinergic receptors on the excitability of corticothalamic layer VI pyramidal neurons using whole-cell recordings in acute brain slices of the prefrontal cortex. We focus in particular on how cholinergic excitation of these layer VI neurons is altered by genetic deletion of either the α5 or β2 nicotinic receptor subunits. We find that mice lacking the α5 subunit have significantly reduced nicotinic receptor mediated responses to acetylcholine, whereas the response is absent in mice lacking the β2 subunit. However, despite showing similar differences across genotypes in terms of the magnitude of cell depolarization, the increase in action potential firing observed in response to acetylcholine application is not weakened in α5 or β2 knockout mice when both nicotinic and muscarinic receptors are activated. As suspected, muscarinic receptor mediated responses to acetylcholine in α5 and β2 knockout mice are significantly enhanced when compared to wild type mice. This change is mediated by a functional increase in muscarinic M1 receptor signaling, with a small muscarinic M3 receptor component in most layer VI pyramidal neurons. Our findings suggest that disrupting nicotinic receptor function can fundamentally alter the mechanisms and timing of excitation in prefrontal attentional circuitry. Ongoing work will attempt to pharmacologically dissect downstream mechanisms contributing to the observed muscarinic plasticity.

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