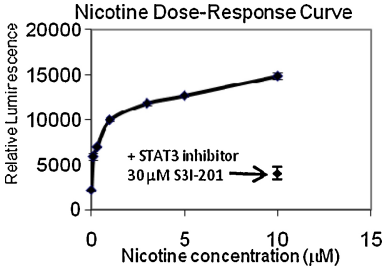
cells transfected with the reporter plasmid but lacking nicotinic receptors. In contrast, co-transfection with α7 in GH4-C1 cells allows dose-dependent nicotine-driven Stat3 signaling that is blocked by 30 μM Jak inhibitor AG-490 or 30 μM Stat3 inhibitor S3I-201. In order to see nicotine-driven α7-dependent 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