adult male SD rats received 6 mg/kg/day via osmotic mini pump for 14 days and were sacrificed immediately after nicotine treatment or after an additional 30 days without treatment. Four brain punches of the VTA were taken from each animal and mRNA was hybridized to Affymetrix Rat Genome 230 2.0 arrays. Two-way ANOVA of age and treatment was performed with 10% FDR using Partek Genomics Suite. We identified three classes of differentially expressed genes including transient, persistent, and late phase genes. There were a total of 267 transient phase genes (80 adol specific, 176 adult specific, 11 shared), 106 persistent phase genes (63 adol specific, 34 adult specific, 9 shared), and 1011 late phase genes (546 adol specific, 103 adult specific and 362 shared). Ontological analysis revealed a number of overrepresented classes of genes regulating nervous system development and function specific to adolescent nicotine exposure. These include 43 genes regulating neurite development, growth and morphology. Other genes of interest specific to adolescent nicotine exposure were 6 genes regulating circadian rhythms, and 23 genes involved in schizophrenia. Furthermore, these genes form an extensive interaction network, whereas those genes specific to the adult form no network. Lastly, network analysis revealed significant regulation of the synaptic long term potentiation canonical pathway in the adolescent treatment group. This suggests chronic nicotine causes large scale changes in plasticity in the adolescent brain not seen in the adults. Further examination of these genes may help reveal the underlying causes of the observed increased vulnerability of adolescent smokers.

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3.2

Nicotine persistently activates prefrontal layer VI pyramidal neurons through α5 subunit-containing α4β2* nicotinic acetylcholine receptors

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We have recently shown that corticothalamic neurons in layer VI of prefrontal cortex are excited by nicotinic receptor stimulation during development [1]. These neurons are the major source of corticothalamic feedback projections and play a key role in attention. Yet, it is not well understood how layer VI neurons are affected by acetylcholine and nicotine in adulthood. Human imaging work has shown that nicotine from one cigarette saturates cortical nicotinic receptors for several hours [2]. This finding has been widely interpreted to suggest that smoking results in the inactivation of cortical nicotinic receptors through desensitization. However, it is unclear that nicotine would equally desensitize all subtypes of α4β2* nicotinic receptors in the cortex. In particular, the properties of nicotinic receptors may be altered by the presence of the α5 accessory subunit (encoded by the CHRNA5 gene). Here, we investigate the effects of nicotinic stimulation on layer VI pyramidal neurons in adult mice. Since both acetylcholine (1 μM to 1 mM) and nicotine (300 nM) can elicit significant inward currents in layer VI neurons of wildtype mice, we tested the contribution of the nicotinic receptor α5 subunit by examining these responses in mice in which this receptor subunit has been genetically deleted [3]. Layer VI neurons in these α5−/− mice showed a maximal inward current with acetylcholine which was approximately one third of that observed in α5+/+ mice. In both genotypes, the cholinergic currents were recorded in the presence of 200 nM atropine, were reversibly inhibited by the α4β2*-selective antagonist DHβE (3 μM), and were resistant to 2 μM TTX, suggesting that they are mediated directly by receptors on the recorded cells. Similar to our findings with acetylcholine, 300 nM nicotine elicited a persistent inward current in α5−/− mice which was approximately one third of that in α5+/+ mice. Interestingly, this application of nicotine had a significantly greater ability to desensitize layer VI neurons to subsequent application of acetylcholine in α5−/− mice compared with α5+/+ mice. Results from this study suggest that the presence of nicotinic receptor α5 subunits in layer VI neurons is necessary for their normal response to acetylcholine, contributes significantly to their persistent activation by nicotine and protects against the desensitizing effects of nicotine. Prolonged binding of nicotine to prefrontal layer VI nicotinic receptors following cigarette smoking likely has sustained effects on attention gating through corticothalamic pathways because of their expression of the nicotinic receptor α5 subunit.

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3.3

Distinct pharmacological profiles for nicotinic AChR-evoked noradrenaline release in rat frontal cortex and hippocampus

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Nicotinic acetylcholine receptors (nAChRs) are widely distributed in the mammalian brain and modulate many neurotransmitter systems. Noradrenaline (NA) is important for spatial learning in hippocampus (HC) and alertness/attention in frontal cortex (FC). The modulation of NA release by nAChRs has been extensively studied in the HC, showing that NA release in this area is predominantly governed by α3β4* and α7 nAChRs [1]. Here, we compare the effects of nicotinic agonists on [^{3}H]NA release from FC prisms, using a 96 well filtration assay, and report distinct differences in the regulation of [^{3}H]NA release compared with HC. In FC, nicotine and the β2* nAChR-selective agonist 5-I-A-85380 elicit [^{3}H]NA release (EC_{50} = 0.78 μM and 5.8 nM respectively) and these responses are blocked by β2* nAChR antagonist DHβE. In contrast, in the HC these agonists are less potent (EC_{50} > 10 μM nicotine and >0.1 μM 5IA) but more efficacious. These responses are insensitive to DHβE, in agreement with previous findings [2]. Furthermore, [^{3}H]NA release from the FC is insensitive to the α7 nAChR agonist choline, which is effective in releasing [^{3}H]NA from HC prisms, via an indirect action. Responses to cytisine also differed between these regions. Thus in contrast to the HC, β2* nAChRs in the FC are implicated in modulating NA release, but α7 nAChRs are not involved. This distinction may reflect the two populations of noradrenergic neurons that have

been characterized in the locus coeruleus with respect to their complement of nAChR subunit mRNA [3]. To investigate if the nAChR subtypes involved in NA release are affected by chronic nicotine or withdrawal, rats received 0.4 mg/kg nicotine sc once daily for 14 days ± 3 days withdrawal. [^{3}H]NA release in response to 5IA, nicotine and choline was compared in FC and HC prisms in vitro. No differences from saline treated animals were detected in either chronically treated or withdrawn rats in either region examined. This result contrasts with the increase in choline-evoked [^{3}H]NA release in HC prisms following 3 days of withdrawal from nicotine administered via osmotic minipump [1]. This suggests differing responses to sustained and intermittent nicotine administration.

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3.4

Functional interaction between presynaptic nicotinic and D2 receptors on dopaminergic nerve endings of rat and mouse nucleus accumbens

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It is well known that cross-talk between receptors represent an important mechanism of neurotransmission modulation and plasticity. Although these interactions have been mostly localized postsynaptically, receptor cross-talk which involve common intracellular pathways have been reported to occur also at the presynaptic level [1–3]. Neuronal nicotinic acetylcholine receptors (nAChRs) in the CNS are located mostly presynaptically and have been implicated in facilitating release of neurotransmitters [4]. It has been shown that dopaminergic axon terminals in the nucleus accumbens possess nAChRs mediating enhancement of dopamine (DA) release. We investigated whether nAChRs and DA autoreceptors interact on the same nerve endings using rat and mouse nucleus accumbens (NAc) synaptosomes prelabeled with [^{3}H]DA and exposed to nicotinic and dopaminergic receptor ligands. The nicotinic agonists (-)nicotine or epibatidine provoked [^{3}H]DA release which was inhibited by quinpirole. This effect was blocked by sulpiride and raclopride. The [^{3}H]DA overflow evoked by 4-aminopyridine (4-AP) was markedly inhibited by quinpirole. This inhibitory effect did not change either in absence or in presence of (−)nicotine when the nAChRs were desensitized. The inhibitory effect of quinpirole disappeared after a preincubation with this drug. However, the stimulatory effect of (−)nicotine did not change when the DA autoreceptors were desensitized. (−)Nicotine and 4-AP were able to stimulate [^{3}H]DA overflow also in mouse synaptosomes and this overflow was partially inhibited by quinpirole. In the nAChR subunits β_2 knockout mice the (−)nicotine-evoked [^{3}H]DA overflow was abolished but quinpirole was still able to inhibit the [^{3}H]DA overflow elicited by 4-AP. In conclusion, the (−)nicotine evoked-release can be modulated by D_2/D_3 autoreceptors present on the DA terminals and nAChRs function is independent from the activation of D_2/D_3 autoreceptors which themselves may function independently from the activation of presynaptic nAChRs.

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3.5

Effects of nicotine on real-time dopamine dynamics in rat nucleus accumbens: *In vivo* voltammetric study

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Nicotine, a primary component of tobacco, is one of the most abused drugs worldwide. Success in developing effective treatments for smoking cessation depends on a detailed understanding of the neurochemical changes caused by nicotine in the brain. For many years it has been known that the increase in extracellular dopamine concentration in the nucleus accumbens, a key reward region in the brain, is related to addictive effects of nicotine as well as other drugs of abuse. However, many questions remain unanswered in regard to nicotine-induced changes in the delicate equilibrium between dopamine release and uptake within neurotransmission events that happen on a subsecond timescale. The present study evaluated the effects of nicotine on dopamine release and uptake in the nucleus accumbens of anesthetized rats using fast-scan cyclic voltammetry. The time (ms) and spatial (μm) resolutions of this technique allow a detailed examination of the kinetics of dopamine release and uptake, eliminating the potential contribution of its metabolism. We found that nicotine injection (0.03, 0.1 and 0.3 mg/kg, i.v.) dose-dependently induced dopamine efflux 5–7 s after drug administration. The maximum dopamine concentration was 0.82 ± 0.08 μM. This effect is likely due to nicotine-induced increases in VTA neuron burst firing which has previously been demonstrated in electrophysiological studies. In contrast, nicotine reduced accumbal dopamine release in response to electrical stimulation of the VTA (24 rectangular pulses, 60 Hz, 300 μA, 2 ms/phase). However, no changes in dopamine uptake parameters were detected. The decrease in amplitude of the electrically evoked dopamine signal by nicotine may be due to engagement of dopamine autoreceptors in response to the increased cell firing rate and accumulated extracellular dopamine concentration in the nucleus accumbens. This effect is consistent with observations of nicotine's effect on dopamine in microdialysis studies. Results from this study provide new insight into the acute nicotine-induced changes associated with dopamine release and uptake at the level of presynaptic terminals.

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