Cellular and Synaptic Mechanisms of Nicotine Addiction

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ABSTRACT: The tragic health effects of nicotine addiction highlight the importance of investigating the cellular mechanisms of this complex behavioral phenomenon. The chain of cause and effect of nicotine addiction starts with the interaction of this tobacco alkaloid with nicotinic acetylcholine receptors (nAChRs). This interaction leads to activation of reward centers in the CNS, including the mesoaccumbens DA system, which ultimately leads to behavioral reinforcement and addiction. Recent findings from a number of laboratories have provided new insights into the biologic processes that contribute to nicotine self-administration. Examination of the nAChR subtypes expressed within the reward centers has identified potential roles for these receptors in normal physiology, as well as the effects of nicotine exposure. The high nicotine sensitivity of some nAChR subtypes leads to rapid activation followed in many cases by rapid desensitization. Assessing the relative importance of these molecular phenomena in the behavioral effects of nicotine presents an exciting challenge for future research efforts. © 2002 Wiley Periodicals, Inc. J Neurobiol 53: 606–617, 2002

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INTRODUCTION

Tobacco use is a major public health problem worldwide, and the numbers of smoking-related deaths are second only to malaria (WHO, 1999). Nearly one-third of adults worldwide are smokers, and the majority started the habit as adolescents. About half of those who smoke through adulthood will die from smoking-related diseases (WHO, 1997). Sadly, the number of smokers continues to increase in developing nations, where availability and marketing of tobacco products has increased faster than public health education (Peto et al., 1992, 1999). These statistics highlight the fact that tobacco is a strong motivator of a very unhealthy behavior.

NICOTINIC RECEPTORS AND ADDICTION

Addiction is a complex behavioral phenomenon with causes and effects that range from molecular mechanisms to social interactions. Ultimately, the process of drug addiction begins with molecular interactions that alter the activity and metabolism of the neurons that are sensitive to that drug. Over time, this alters the properties of individual neurons and circuits, which leads to complex behaviors such as dependence, tolerance, sensitization, and craving (Koob et al., 1997;

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Nestler and Aghanjanian, 1997). In the case of tobacco products, the principal addictive component is nicotine, which interacts with specific membrane receptors in the nervous system known as neuronal nicotinic acetylcholine receptors (nAChRs).

A common feature of many addictive drugs, including nicotine, is that they increase dopamine (DA) levels in the nucleus accumbens (NAcc) at the same concentrations that are achieved in serum during self-administration (Stolerman and Jarvis, 1995; Dani and Heinemann, 1996; Picciotto et al., 1998; Dani and De Biasi, 2001). The principal dopaminergic projections to the NAcc arise from neurons in the VTA (Fig. 1). Evidence that NAcc DA levels are important in reward has come from VTA lesion studies and microperfusion of the NAcc with DA receptor antagonists, both of which result in reduced self-administration of many addictive drugs, including nicotine (Balfour, 1991; Corrigall and Coen, 1991; O'Neill et al., 1991; Corrigall et al., 1992, 1994; Vezina et al., 1994; Museo and Wise, 1995; Louis and Clarke, 1998). Although some drugs of abuse alter DA metabolism or reuptake to increase DA levels in the NAcc, nicotine alters the activity of VTA neurons to enhance DA release. It should be noted that there are important differences in cellular and behavioral effects of changing DA levels by these two mechanisms. Interestingly, nicotine appears to preferentially stimulate activity in and release from DA neurons in the mesoaccumbens but not the nigrostriatal system, despite the fact that these cells have many other properties in common (Imperato et al., 1986; Mereu et al., 1987; Benwell and Balfour, 1997).

Although there is strong evidence linking NAcc DA levels and reward, several recent studies suggest that this may be indirect. A more complex and less direct role for DA has been hypothesized, suggesting that DA signals novelty or reward expectation rather than reward itself (Berke and Hyman, 2000; Schultz et al., 1997; Dani et al., 2001; DiChiara, 2000). In a recent study, rats were equipped with intracranial self-stimulation devices in midbrain dopamine areas. In these individuals, self-stimulation of the reward centers caused elevation in NAcc DA levels during the learning period, but these increases were not seen in response to self-stimulation even 30 min later (Garris et al., 1999). Thus, regulatory processes appear to control DA release. The inhibition of action-potential driven DA release in the striatum by physiologically relevant nicotine concentrations suggests that cholinergic mechanisms may be important in these control mechanisms (Zhou et al., 2001).

Although addiction likely involves the convergence of many CNS effects, the importance of the DA system provides a focus for many studies and for this review. We will outline recent findings that address the cellular effects associated with the first exposure to nicotine. Although these data are clearly relevant to the initiation of nicotine addiction, they may also be

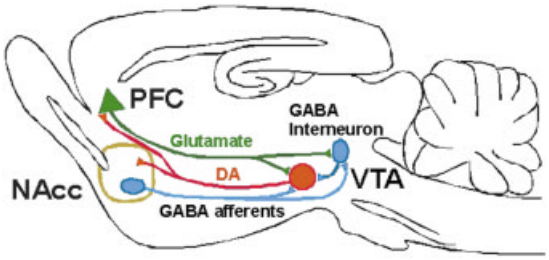

Figure 1 A simplified diagram of the glutamatergic and GABAergic inputs to the ventral tegmental area (VTA). NAcc nucleus accumbens, PFC prefrontal cortex, DA dopamine, GABA gamma-amino butyric acid.

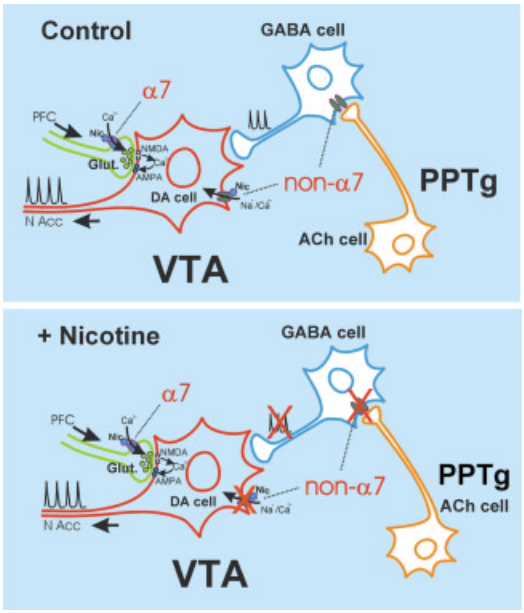

Figure 2 A schematic of the role of nAChRs in the control of VTA DA neuron excitability. Under control conditions (upper panel) non-α7 nAChRs can excite DA and GABA neurons directly, while α7 receptors can enhance release from glutamatergic terminals. Endogenous ACh release from brainstem cholinergic neurons contributes to the GABAergic input to VTA DA neurons. In the presence of nicotine concentrations similar to those found in a smoker's blood (lower panel), the non-α7 nAChRs desensitize rapidly, effectively inhibiting GABAergic inputs to the DA neurons. The α7 nAChRs will not desensitize as much, which means that glutamatergic inputs will be enhanced as the GABAergic inputs are depressed, thus leading to a net increase in excitation of the DA neurons.

relevant to the more complex phenomena associated with continued use of the drug.

We know that nicotine influences neuronal activity, synaptic communication, and ultimately behavior, through its effects on nicotinic receptors. These receptors are pentameric membrane proteins that include two or more agonist binding sites and a central aqueous pore. Agonist binding results in a conformational change that leads to ion flux through the pore, inducing a depolarization and increased excitability. Pharmacologic and ligand-binding studies have demonstrated considerable diversity in neuronal nAChR subtypes. To date, 12 nAChR subunit genes have been identified, $\alpha 2$–$\alpha 10$ and $\beta 2$–$\beta 4$ (Heinemann et al., 1990; Sargent, 1993; McGehee and Role, 1995; Lindstrom, 1996; Elgoyhen et al., 1994, 2001). The contribution of specific subunits to cellular responses can be accomplished to a large degree with the use of selective agonists and antagonists. However, as much of this pharmacologic information has come from heterologous expression studies, one must be cautious when inferring nAChR structure of native receptors from drug sensitivity. With this caveat in mind, selective ligands can be used to indicate the contribution of specific subunits to native receptor responses (McGehee and Role, 1995; Lindstrom, 1996).

Important functional properties of these receptors that contribute to their physiologic effects include activation, desensitization, and upregulation following nicotine exposure. There is considerable diversity in the sensitivity of different receptor subtypes to nicotine. Different affinities also lead to differences in channel activation and subsequent desensitization in the continued presence of the drug. Nicotinic receptor upregulation is a perplexing phenomenon whereby the receptor sensitivity and binding levels increase following nicotine preexposure for just a few hours. Each of these phenomena is likely to contribute to the behavioral reinforcement by nicotine, but the relative importance of each is not known (Dani and Heinemann, 1996).

NICOTINIC RECEPTOR UPREGULATION

Upregulation of nAChR function and ligand binding following preexposure to nicotine varies with cell type and receptor subtype (Schwartz and Kenneth, 1985). Physiologically relevant nicotine concentrations have been shown to upregulate $\alpha 4\beta 2$-containing receptors (e.g., Flores et al., 1991; Buisson and Bertrand, 2001). Upregulation of other receptor subtypes can occur with higher nicotine concentrations in some cells (e.g., Schwartz and Kenneth, 1985; Rogers and Wonnacott, 1997; Molinari et al., 1998). Nicotinic receptor upregulation has previously been reported to involve an increase in the number of receptors, but this is not associated with changes in mRNA and is thought to reflect increased assembly (Olale et al., 1997; Wang et al., 1998). Recent studies suggest that the increased binding following upregulation may reflect a change in receptor state rather than receptor number (W. Green personal communication).

In association with the upregulation of ligand binding following nicotine preexposure, some laboratories report increases in nicotinic responses (Ksir et al., 1987; Clarke et al., 1988; Rowell and Wonnacott 1990; Yu and Wecker 1994; Buisson et al., 2000; Buisson and Bertrand, 2001), while others have found decreases in function (Marks et al., 1985, 1993; Lapchak et al., 1989). Differences in assays and treatment paradigms may explain some of this variability, but these mixed effects complicate the formulation of a reasonable prediction of the effects of nicotine self-administration on nAChR responses. It is reasonable to hypothesize that nicotine self-administration will enhance nAChR responses based upon the observation that preexposure to nicotine can sensitize animals to its locomotor and self-administration effects (Wise and Bozarth, 1987; Shoaib et al., 1997). It remains to be shown, however, whether nicotine self-administration induces receptor upregulation *in vivo*, although there are intriguing reports of high [^{3}H]-nicotine binding in brain tissue from postmortem smokers (Breese et al., 1997; Court et al., 2000; Patterson and Nordberg, 2000).

SYNAPTIC TRANSMISSION IN THE MESOACCUMBENS DOPAMINE SYSTEM

As outlined above, the links between drug self-administration and NAcc DA levels have motivated many investigations into the factors affecting excitability of VTA DA neurons (Dani et al., 2001; Dani and De Biasi, 2001). The principal excitatory inputs to the VTA DA neurons are glutamatergic projections from the prefrontal cortex (Fig. 1; Kalivas et al., 1989; Johnson et al., 1992; Sesack and Pickel, 1992; Suaud-Chagny et al., 1992; Taber et al., 1995; Carr and Sesack, 2000). The principal inhibitory inputs to VTA neurons are GABAergic, including local interneurons as well as projections from NAcc and the ventral pallidum (Kalivas et al., 1993). Cholinergic projections to the VTA come from brain stem nuclei, the pedunculopontine tegmental nucleus (PPTg) and the

lateral dorsal tegmental nucleus (LDTg). Ultrastructural analyses have shown that cholinergic boutons within the VTA contact postsynaptic structures with low levels of dopamine transporter expression (Garzón et al., 1999). Numerous other neurotransmitters and neuromodulators influence the activity of the VTA, including serotonin, norepinephrine, endogenous opioids, and others (Tzschentke, 2001). Although this review focuses on GABA and glutamate inputs to VTA DA neurons, it is important to consider many cellular interactions in this system.

DA release from VTA projections is ultimately due to the balance of excitatory and inhibitory inputs and the intrinsic activity of the DA neurons. Nicotinic receptors of various subtypes are expressed by DA neurons, GABA neurons, and by the axon terminals of glutamatergic inputs to this nucleus. This review will focus on recent work from our lab and others showing that an important physiologic role for these receptors is the modification of synaptic transmission within the VTA.

VTA nAChRs AND BEHAVIOR

Just as a large portion of the global human population is drawn to tobacco use on a daily basis, rodents readily self-administer nicotine when the opportunity is presented in the laboratory. Despite the widespread expression of nAChRs throughout the brain, the nAChRs found in VTA are critically important in the rewarding effects of nicotine (Schilstrom et al., 1998b; Nisell et al., 1994). When the nAChR antagonist mecamylamine (MEC) is focally infused in the VTA through a microdialysis probe, the increase in extracellular DA in the NAcc caused by a systemic injection of nicotine is blocked. Infusion of MEC in the NAcc does not prevent the DA increase (Nisell et al., 1994). Similarly, nicotine self-administration in rats diminishes when the nAChR antagonist dihydro-β-erythroidine (DHβE) is infused into the VTA. Self-administration is not affected by infusion of DHβE in the NAcc (Corrigall et al., 1994). Thus, while systemic administration of nicotine affects nAChRs in many brain areas, including NAcc, hippocampus, and cortex, it is the nAChRs within the VTA that mediate the rewarding effects of nicotine.

To date, three cell types in the VTA have been shown to express nAChRs: Dopamine neurons, GABA neurons, and glutamatergic presynaptic terminals that synapse onto dopamine neurons. VTA DA neurons express mRNAs for many different nAChR subunits. Within the DA neuron population there is variation in the prevalence and amount of nAChR subunit expression, but α2–α7 and β2–β4 mRNAs are all expressed by these neurons (Charpantier et al., 1998; Klink et al., 2001). They give rise to three pharmacologically identifiable nAChRs, one that is likely a homomeric α7 receptor and two that do not contain α7. A majority of DA neurons express nAChRs that can be blocked by MEC at concentrations that block non-α7 containing nAChRs selectively, whereas less than half of the DA neurons express nAChRs containing α7 (Pidoplichko et al., 1997; Klink et al., 2001).

GABA neurons in the VTA express a similar variety of nAChR subunit mRNA, but in contrast to DA neurons, α5, α6, β3, and β4 were found in less than 25% of the GABA neurons, and α2 was not found at all (Klink et al., 2001). Thus, the majority of the GABA neurons in the VTA express nAChRs that most likely contain α4 and β2 subunits, which are also blocked by MEC or DHβE (Mansvelder et al., 2002).

As mentioned above, the VTA receives glutamatergic synaptic input primarily from the prefrontal cortex. This input has been suggested to provide the major excitatory control of VTA neuron activity and ultimately DA release in the NAcc (Kalivas et al., 1989; Johnson et al., 1992; Sesack and Pickel, 1992; Suaud-Chagny et al., 1992; Taber et al., 1995). Recently, Carr and Sesack (2000) reported that glutamatergic projections from PFC do not synapse onto DA neurons that project to the NAcc. Rather, they found contacts onto GABAergic projection neurons and DA neurons that project back to PFC. Thus, other glutamatergic inputs appear to be responsible for the direct excitation of the mesoaccumbens DA projections. One intriguing possibility is that glutamate release within the VTA may come from DA neurons themselves as has been demonstrated *in vitro* (Sulzer et al., 1998).

Independent of the origins of the glutamatergic inputs to VTA DA neurons, focal administration of the NMDA receptor antagonist APV within the VTA *in vivo* inhibits nicotine-induced increases in DA release within the NAcc (Schilstrom et al., 1998a), suggesting that nicotinic modulation of glutamatergic transmission contributes to the enhancement of VTA DA output. Thus, the presynaptic terminals of the glutamatergic inputs to mesoaccumbens DA neurons most likely express nAChRs. In brain slice recordings from VTA DA neurons, glutamatergic transmission onto these neurons is enhanced by low concentrations of nicotine. This enhancement is unaffected by TTX, which blocks action potential firing, suggesting that the nAChRs mediating this effect are situated locally in the VTA, on the presynaptic glutamatergic termi-

nals (Mansvelder and McGehee, 2000). These nAChRs are sensitive to MLA, a selective inhibitor of nAChRs that contain the $\alpha 7$ subunit (Alkondon and Albuquerque, 1993; Seguela et al., 1993). *In vivo* focal injection of MLA into the VTA also prevents nicotine-induced increases in accumbal DA release (Schilstrom et al., 1998b). Nicotinic AChRs consisting of $\alpha 7$ subunits are well-suited for modulating synaptic transmission as they have a high calcium permeability, and this calcium flux occurs at resting membrane potentials when activated by agonists (Seguela et al., 1993; McGehee and Role, 1995).

The nicotine concentration profile in a smoker's blood during cigarette smoking is very different from ACh concentration profiles in a cholinergic synapse. In the cholinergic synapse, the ACh concentration rises within a millisecond to millimolar concentrations (Kuffler and Yoshikami, 1975). During cigarette smoking, blood nicotine levels reach 300–500 n*M* several minutes after the initiation of smoking and concentrations close to 250 n*M* are sustained for 10 min or more (Henningfield et al., 1993; Gourlay and Benowitz, 1997). The high-affinity nAChRs including $\alpha 4\beta 2$ and $\alpha 3\beta 2$ subtypes have measurable activity at these nicotine concentrations. These slow concentration profiles are important considerations when examining the effects of nicotine on receptor activation as well as desensitization.

The low concentrations of nicotine experienced by smokers activate high-affinity nAChRs on VTA DA neurons (Calabresi et al., 1989; Pidoplichko et al., 1997; Picciotto et al., 1998). Following activation, even with nicotine concentrations as low as 100–500 n*M*, the somatic nAChRs desensitize within minutes (Pidoplichko et al., 1997; Fisher et al., 1998; Dani et al., 2000). However, at the same time, *in vivo* biochemical studies show that a single systemic injection of nicotine enhances DA release in the NAcc for more than an hour (Di Chiara and Imperato, 1988; Di Chiara, 2000; Imperato et al., 1986; Schilstrom et al., 1998a; Schilstrom et al., 1998b). Clearly, there must be additional mechanisms that follow nAChR activation, which induce the long-term enhancement of DA release.

We have recently identified two synaptic mechanisms in the VTA by which nicotine has a long-lasting stimulatory effect on the VTA DA neuron which outlasts nAChR desensitization: Nicotine-induced long-term potentiation (LTP) of the excitatory glutamatergic input and nicotine-induced depression of GABAergic transmission (Mansvelder and McGehee, 2000; Mansvelder et al., 2002).

NICOTINIC MODULATION OF GLUTAMATERGIC TRANSMISSION IN THE VTA

VTA DA neurons have signature electrical properties that help in their identification. These neurons fire spontaneously and are known to express pacemaker currents, or hyperpolarization induced currents (I_h; Johnson and North, 1992; Pidoplichko et al., 1997). Administration of nicotine and other drugs of abuse have been shown to induce a burst type firing pattern *in vivo*, which is apparently necessary for the enhancement of DA release within the NAcc (Suaud-Chagny et al., 1992; Murase et al., 1993). Burst firing is dependent upon NMDA receptor activation (Johnson et al., 1992), providing a potential link between behavioral reinforcement and LTP induction in the VTA. The glutamatergic input to the DA neurons can undergo LTP in response to pairing of pre- and postsynaptic stimulation, and this process is dependent on NMDA receptor activation (Bonci and Malenka, 1999). Interestingly, the excitatory inputs to the GABAergic interneurons within this nucleus do not express LTP following identical induction protocols.

Nicotinic receptors are present on both the presynaptic glutamatergic terminals and on the DA neurons. When nicotine arrives in the VTA, it stimulates both glutamatergic terminals and DA neurons directly, which can mimic the paired electrical stimulation of the pre- and postsynaptic partners. Thus, potentiation of excitatory transmission in the VTA is favored by the presence of nicotine. In our experiments, nicotine could replace presynaptic stimulation completely in LTP induction. Although we found no evidence for postsynaptic nAChR contribution to LTP, this was tested under conditions where nicotine was the only stimulus to the postsynaptic neuron. It is likely that the magnitude of postsynaptic stimulation necessary to induce LTP is lower during nAChR activation, but this remains to be tested in the VTA. In hippocampal slice recordings, Ji et al. (2001) found that focal application of ACh to the dendritic region of CA1 pyramidal neurons leads to LTP induction by a weak stimulus that only induces STP without nAChR activation. In addition, focal ACh application induces persistent enhancement of glutamatergic contacts between medial habenula and interpeduncular neurons (Girod and Role, 2001). Together, these findings highlight cellular mechanisms that may contribute to the prolonged excitatory effects on DA release following nicotine exposure (Mansvelder and McGehee, 2000). Presynaptic $\alpha 7$ nAChRs provide a rapid means

to increase intracellular calcium presynaptically, as these channels will gate at the resting membrane potential (Seguela et al., 1993). Simultaneous activation of the nAChRs on postsynaptic DA neurons will increase the likelihood of NMDA receptor activation due to depolarization and relief of magnesium block. In mutant mice lacking the β2 subunit, which normally contributes to the majority of nAChRs on DA neurons, there is no long-term activation of the DA system by nicotine, and the mice do not self-administer nicotine (Picciotto et al., 1998). This suggests that without β2-containing nAChRs, nicotine does not depolarize the DA neuron enough to relieve magnesium block of NMDA receptors.

The nicotine-induced LTP may contribute to the time course of DA release in the NAcc following a single systemic injection of nicotine (Schilstrom et al., 1998a). Surprisingly, LTP is induced at very low concentrations of nicotine, in the range of that experienced by smokers, and an exposure time of 200 s is sufficient. Thus, it is very likely that synaptic plasticity in the VTA is already induced by amounts of nicotine that a person would get in his or her brain after smoking of one cigarette. In addition, these findings suggest that cellular mechanisms thought to contribute to learning and memory can be activated by a drug of abuse (Mansvelder and McGehee, 2000). Similarly, Bonci, Malenka, and colleagues have shown recently that a single administration of cocaine to neonatal rats can induce LTP of excitatory inputs to VTA DA neurons that persists for up to 10 days (Ungless et al., 2001). Together, this work identifies physiological correlates of a growing body of evidence that drugs of abuse can activate memory mechanisms within the brain reward centers (Nestler, 2001).

NICOTINIC MODULATION OF GABAERGIC TRANSMISSION IN THE VTA

In addition to excitatory inputs, VTA DA neurons are under inhibitory control, predominantly by GABAergic inputs. GABAergic inputs to the VTA DA neurons come from local interneurons and from projection fibers from the NAcc and the ventral pallidum (Walaas and Fonnum, 1980; Kalivas et al., 1993; Steffensen et al., 1998). When nicotine reaches the VTA, nAChRs expressed by GABA neurons in the nucleus are activated and cause an increase in the firing rate of these neurons (Yin and French, 2000; Mansvelder et al., 2002). These nAChRs are mostly of the non-α7 type that likely contain α4 and β2 subunits. When nicotine is applied to these receptors there is a transient increase in inhibitory input to DA neurons in the VTA. This effect would likely offset some of the excitatory effects of nicotine during the time of enhanced GABA transmission.

Similar to the non-α7 nAChRs on the DA neurons, the nAChRs on the GABA neurons also desensitize rapidly. Thus, the increased activity of the GABA neuron subsides and the inhibitory input to the DA neurons diminishes. Desensitization not only prevents further activation of nAChRs by nicotine, it also precludes the contribution of those nAChRs to endogenous cholinergic signaling. Cholinergic inputs to VTA from the laterodorsal and the pedunculopontine tegmental nuclei (Oakman et al., 1995) selectively target non-DA neurons and a subset of DA neurons (Garzón et al., 1999). VTA DA neurons are only sparsely targeted by cholinergic projections. While practically all DA neurons in the VTA express nAChRs (Pidoplichko et al., 1997), only 5% of the neurons actually receive cholinergic projections (Fiorillo and Williams, 2000). This cholinergic control of inhibitory interneurons is similar to evidence showing direct cholinergic innervation of hippocampal interneurons (Frazier et al., 1998a, 1998b). The functional role of the nonsynaptic nAChRs on DA neurons remains mysterious, although they may contribute to intercellular communication via nonsynaptic "volume" transmission (Umbriaco et al., 1995; Zoli et al., 1998). Preventing the breakdown of ACh in brain slices that include the VTA with an inhibitor of cholinesterase increases the excitability of GABA neurons similar to the effect of nicotine perfusion (Mansvelder et al., 2002). This supports the idea that there is functional cholinergic input to the VTA, and that it can affect GABA transmission.

Endogenous cholinergic drive to VTA GABA neurons can be inhibited either by the application of nAChR antagonists, or by desensitization due to nicotine exposure. In either case, the loss of nAChR activity in the slice not only inhibits the stimulatory effect of nicotine on GABA activity, but in the majority of the GABA neurons, also leads to a reduction of activity below baseline (Mansvelder et al., 2002). As a result, DA neurons in the VTA receive less inhibitory GABAergic input than before nicotine arrived in the VTA, and this decrease of inhibitory tone results in increases in action potential firing.

The nAChRs on the GABA neurons recover very slowly from desensitization. In the first 15 min after nicotine is present, GABA neurons do not respond at all to a next nicotine application. After that, the response recovers slowly, taking approximately 1 h to reach normal levels of nicotine sensitivity (Mans-

velder et al., 2002). The recovery of endogenous cholinergic transmission would require a similar time course. During this recovery phase GABA neurons are less active, and DA neurons receive less inhibitory input, making them more active.

Although α7 nAChRs on the glutamatergic terminals also desensitize rapidly, the low nicotine concentrations associated with tobacco use induce much less desensitization of these receptors. A 10-min exposure to 250 n*M* nicotine completely desensitizes the nAChRs on GABA neurons. During a similar nicotine treatment, the enhancement of glutamatergic transmission does not show significant desensitization. Thus, excitatory inputs to VTA DA neurons are enhanced by nicotine while inhibitory GABAergic inputs are depressed (Fig. 2). In addition, if the DA neuron is depolarized sufficiently, the enhancement of glutamatergic transmission can induce a long-term potentiation of these inputs. The DA neurons receive a net increase in excitatory drive from the synaptic inputs that outlasts both the presence of nicotine and the time course of nAChR activation. In sum, through the differential expression of nAChR subtypes by DA neurons, GABA neurons and glutamatergic terminals in the VTA, activation and desensitization can act together to increase DA neuron activity in a persistent manner. The subcellular localization of nAChRs with different properties leads to a persistent enhancement of DA release from the afferent terminals of VTA DA neurons in the NAcc in response to a single exposure to nicotine, as is found *in vivo*.

Nicotinic AChRs have been reported to modulate excitatory glutamatergic transmission in several brain regions (Vidal and Changeux, 1993; McGehee et al., 1995; Gray et al., 1996; Alkondon et al., 1996; Wonnacott, 1997; Mansvelder and McGehee, 2000). In addition, nAChRs can modulate GABAergic transmission in multiple brain areas, such as thalamus, cortex, hippocampus, and interpeduncular nucleus (Lena et al., 1993; Alkondon et al., 1997, 2000; Lena and Changeux, 1997; Fisher et al., 1998; Radcliffe et al., 1999). Modulation of GABA neurons by nAChRs has been most extensively studied in the hippocampus, where GABAergic interneurons express multiple nAChR subtypes (Alkondon et al., 1997; Jones and Yakel, 1997; Frazier et al., 1998b; McQuiston and Madison, 1999; Ji and Dani, 2000). The physiologic impact of nAChR activation is critically dependent upon their localization. There is evidence for nAChR expression both on presynaptic terminals, where they directly modulate GABA release, independent of action potential firing (Fisher et al., 1998; Lu et al., 1999; Radcliffe et al., 1999), and away from the synaptic terminal, where modulation of GABA release is TTX sensitive (Alkondon et al., 1997, 2000; Frazier et al., 1998b). The nAChR-induced modulation of GABAergic transmission in VTA is TTX sensitive, suggesting that the receptors are not expressed on the terminals per se.

Nicotinic receptors on cortical and hippocampal interneurons have been shown to mediate either inhibition or disinhibition of the pyramidal neurons (Alkondon et al., 2000; Ji and Dani, 2000, 2001). nAChR activation in these areas can lead to disinhibition of pyramidal neurons by increasing inhibitory GABAergic transmission to GABA interneurons. Consequently, the pyramidal neurons receive less GABAergic input and are disinhibited. We found that in the VTA, low concentrations of nicotine can also disinhibit DA neurons, but by a different mechanism. Here, nicotine desensitizes nAChRs on GABA neurons, which makes them insensitive to ongoing endogenous cholinergic transmission, thereby reducing GABA neuron excitability. The loss of GABAergic transmission results in a disinhibition of the DA neurons. A similar mechanism may disinhibit hippocampal GABA interneurons (Alkondon et al., 2000). Prolonged exposure to low concentrations of nicotine decreases the ACh sensitivity of the GABAergic input to interneurons. Hippocampal interneurons are one of the few cell types that have been shown to receive cholinergic synaptic input mediated by nAChRs (Frazier et al., 1998a; Hefft et al., 1999). It will be interesting to learn more about nicotine's effects on the contribution of these neurons to the circuitry of the hippocampus.

DISINHIBITION OF VTA DA NEURONS AND REWARD

The depression of GABAergic input to VTA DA neurons by nAChR desensitization occurs following a nicotine exposure similar to that experienced by tobacco smokers. An important question is whether a reduction in VTA GABAergic transmission actually contributes to nicotine addiction. In fact, there is evidence linking GABAergic transmission to behavioral reinforcement. Rats and mice will readily self-administer $GABA_A$ receptor antagonists when they are infused focally into the VTA (David et al., 1997; Ikemoto et al., 1997b). Given the importance of DA release in self-administration, it is not surprising that $GABA_A$ receptor blockade in the VTA also increases DA levels in the NAcc (Ikemoto et al., 1997a; Westerink et al., 1996). Thus, it is likely that the reduction in GABAergic transmission following nAChR desensitization in the VTA contributes to the reinforcing

effects of nicotine. It is important to note that β2 subunits, which mediate the effects of nicotine on GABA neurons, have been shown to be necessary for the maintenance of nicotine self-administration in both rats and mice (Picciotto et al., 1998; Grottick et al., 2000).

Acetylcholinesterase inhibition can enhance GABA transmission in VTA by augmenting endogenous ACh transmission (Mansvelder et al., 2002). In experiments carried out *in vivo,* cholinesterase inhibition in VTA was reported to augment DA release in the NAcc (Blaha et al., 1996). One interpretation of this data is that endogenous ACh transmission in the VTA activates the DA system. However, physiologic experiments indicate very sparse cholinergic inputs to VTA DA neurons (Fiorillo and Williams, 2000), while ultrastructural analyses of cholinergic projections to the VTA found that only a very small proportion of cholinergic terminals make contact on DA neurons (Garzón et al., 1999). The vast majority of the cholinergic neurons in the laterodorsal tegmental and the pedunculopontine nuclei project to GABA neurons in the VTA (Garzón et al., 1999). Therefore, the *in vivo* application of the cholinesterase inhibitor in the VTA by Blaha et al. (1996), which was maintained for several hours, may induce increased DA levels in the NAcc by disinhibition of VTA DA neurons due to nAChR desensitization on GABA neurons.

All tissues tested in our experiments were naïve with respect to nicotine exposure. Thus, GABAergic transmission was modified by a nicotine exposure similar to the levels experienced by a person smoking one cigarette. The depression of GABA transmission was found to outlast the nicotine exposure by many minutes (Mansvelder et al., 2002), while nicotine-induced LTP of excitatory transmission can last for hours or longer (Mansvelder and McGehee, 2000). It is possible that the reduction of GABAergic transmission would also help promote LTP induction, as it would favor depolarization of the DA neurons. Together, these studies emphasize that a limited exposure to nicotine is sufficient to induce lasting changes in the circuitry of the mesolimbic reward system.

DEVELOPMENTAL CHANGES IN NICOTINE SENSITIVITY

For optimal tissue viability and cell visualization our experiments used brain slices from young rats (postnatal days 10–14). There are dramatic changes in the expression of many nAChR subunits during development (Zoli et al., 1995; Broide et al., 1996), which may confound the interpretation of physiologic results. It will be interesting to compare the cellular and synaptic nicotine sensitivity in adult tissue with that seen in tissue from younger animals. Recent studies indicate dramatic differences in the behavioral responses to nicotine between adolescent and adult rats (Faraday et al., 2001). In addition, human adolescents express the initial symptoms of nicotine dependence after smoking of only a few cigarettes (DiFranza et al., 2000). These first symptoms appear during occasional use, before the onset of daily smoking. These behavioral findings support the observations of lasting changes in synaptic activity by a single exposure to nicotine, or to other drugs of abuse, as reported by our laboratory and that of others (Hamid et al., 1997; Vanderschuren et al., 1999; Mansvelder and McGehee, 2000). The synaptic mechanisms that nicotine activates within the DA reward system are likely to underlie the early steps of nicotine dependence.

The potentially dramatic effects of nAChRs on neuronal plasticity are also highlighted by the effects of nicotine exposure during prenatal, postnatal or adolescent development. Nicotine has been reported to alter neuronal morphology, survival, and gene expression in nearly every system that has been examined, including the cholinergic, dopaminergic, serotonergic, and adrenergic systems (Slotkin, 2002).

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