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Long-Term Potentiation of Excitatory Inputs to Brain Reward Areas by Nicotine

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Summary

Nicotine reinforces smoking behavior by activating nicotinic acetylcholine receptors (nAChRs) in the midbrain dopaminergic (DA) reward centers, including the ventral tegmental area (VTA). Although nicotine induces prolonged excitation of the VTA in vivo, the nAChRs on the DA neurons desensitize in seconds. Here, we show that activation of nAChRs on presynaptic terminals in the VTA enhances glutamatergic inputs to DA neurons. Under conditions where the released glutamate can activate NMDA receptors, long-term potentiation (LTP) of the excitatory inputs is induced. Both the short- and the long-term effects of nicotine required activation of presynaptic α7 subunit–containing nAChRs. These results can explain the long-term excitation of brain reward areas induced by a brief nicotine exposure. They also show that nicotine alters synaptic function through mechanisms that are linked to learning and memory.

Introduction

Tobacco use is a leading cause of death in developed countries and is increasing rapidly worldwide (Peto et al., 1992, 1999). The reinforcing effect of nicotine is the primary reason that smoking persists despite the renowned health consequences. Along with other addictive substances such as amphetamines, cocaine, morphine, and heroin, nicotine increases the release of dopamine (DA) in the nucleus accumbens (NAcc) from projections that originate in the ventral tegmental area (VTA) (Di Chiara and Imperato, 1988; Koob, 1992; Nestler, 1992; Nisell et al., 1994; Dani and Heinemann, 1996; Pidoplichko et al., 1997). Concentrations of nicotine that are reached in the blood of smokers (Henningfield et al., 1993) depolarize DA VTA neurons by activating nAChRs on the somata of these neurons (Calabresi et al., 1989; Wada et al., 1989; Clarke, 1993; Pidoplichko et al., 1997; Picciotto et al., 1998). At these concentrations, nAChRs also undergo desensitization within seconds (Pidoplichko et al., 1997), yet nicotine injections in rats enhance DA release in the NAcc for more than an hour (Imperato et al., 1986; Di Chiara and Imperato, 1988; Schilstrom et al., 1998a, 1998b). Thus, apart from the transient activation of somatic nAChRs, additional mechanisms must contribute to the prolonged effects of nicotine.

Glutamatergic synaptic input, primarily from the prefrontal cortex, provides 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). Recent biochemical data indicates that in vivo injection of the glutamate receptor antagonist APV into the VTA largely prevents the stimulatory effect of nicotine (Schilstrom et al., 1998a), suggesting that nicotinic modulation of glutamatergic transmission contributes significantly to the enhancement of VTA DA output. In vivo focal injections of methyllycaconitine (MLA), an inhibitor of α7-containing nAChRs, into the VTA also prevents nicotine-induced increases in DA release (Schilstrom et al., 1998b). These findings, in combination with the observation that presynaptic α7 nAChRs can enhance glutamatergic transmission in other limbic nuclei (McGehee et al., 1995), have led to the hypothesis that nicotine may influence DA release by presynaptic modulation of excitatory input to these neurons (Schilstrom et al., 1998b). Here, we investigate the cellular mechanisms underlying these in vivo observations by studying nicotinic modulation of excitatory synaptic transmission onto VTA DA neurons in brain tissue slices. We show that a short application of a low concentration of nicotine (0.5–1 μM) enhances excitatory synaptic transmission to the VTA and that this effect can outlast nAChR desensitization.

Results

Extracellular stimulation of the tissue rostral to the VTA evoked excitatory postsynaptic currents (EPSCs) that were reversibly blocked by the non-NMDA glutamate receptor antagonist DNQX (Figure 1). When recording from the VTA, DA neurons can be distinguished from GABA neurons, the other major cell type in the VTA, by the presence of a prominent hyperpolarization-activated current (I_h) (Johnson and North, 1992; Mercuri et al., 1995). This study deals only with excitatory synaptic transmission on DA neurons; therefore, the presence of I_h was established at the beginning of each experiment (Figure 2a). In all the recordings, GABAergic transmission was inhibited by bicuculline (20 μM) in the bath solution.

With increasing stimulus strength, the amplitude of the evoked glutamatergic EPSC increased gradually, until it saturated (Figure 2b). Using a submaximal stimulus strength (see Experimental Procedures), bath application of nicotine (1 μM, 2 min) increased the EPSC amplitude to 151% ± 12% of control (Figures 2c–2e; n = 8 of 9). The enhancement outlasted the application, but the EPSC amplitude declined to baseline after several minutes (Figure 2d). In contrast, nicotine did not enhance the evoked EPSC when the stimulus strength was set to evoke the maximal EPSC amplitude (Figures 2f–2h; n = 7). The lack of effect might result from a direct depolarization of the synaptic terminals by the maximal stimulus intensity, thereby precluding nicotinic modulation of transmission. When the distance of the stimulus electrode to the VTA was doubled to 500–600 μm, nicotine (1 μM, 2 min) induced an increase in EPSCs evoked by maximal stimulus in two out of three cells tested (132% ± 5% of control; data not shown; p = 0.02). Although not as pronounced as the modulation

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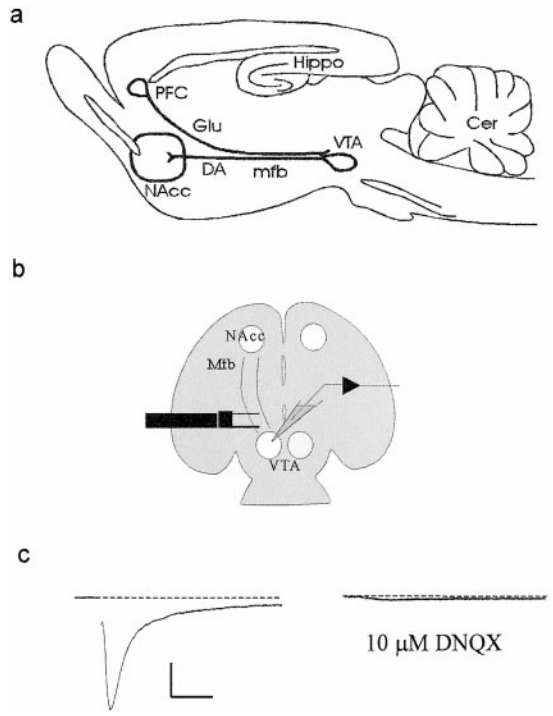

Figure 1. The Brain Reward Areas

(a) Schematic representation of the glutamatergic projections from the prefrontal cortex (pfc) to the VTA and of the DA projections from the VTA to the NAcc. Abbreviations: Mfb, medial forebrain bundle; Cer, cerebellum; Hippo, hippocampus.

(b) Schematic representation of the horizontal brain slice containing the VTA and the NAcc.

(c) The evoked EPSC is blocked by the application of 10 μM DNQX. $V_m = -70$ mV; n = 7. Scale bar: 50 pA, 20 ms.

seen with low stimulus intensity, these findings support the idea that direct depolarization of the glutamatergic terminals by the stimulating electrode can occlude presynaptic enhancement by nicotine.

Presynaptic enhancement of evoked synaptic transmission by nicotine has been shown in other brain regions, and we have assessed whether a similar mechanism explains the modulation of glutamatergic transmission in the VTA. To test whether the site of nicotine-induced enhancement is pre- or postsynaptic, we studied the effect of nicotine on spontaneous EPSCs. The frequency of spontaneous EPSCs varied from cell to cell, but was typically around 1–2 Hz (see examples in Figures 3 and 4). Application of nicotine (1 μM, 2 min) resulted in a statistically significant increase in the frequency of the spontaneous EPSCs in 62% of the DA neurons tested (Figures 3a and 3d; n = 13 of 21). The EPSC frequency typically declined back to control levels after nicotine application, and the total increase lasted 157 ± 23 s. Nicotine also enhanced the baseline noise in the recording (Figure 3a). Noise levels increased from 1.3 ± 0.1 pA in the absence of nicotine to 2.7 ± 0.2 pA in its presence, an increase of 107% ± 18% (n = 21). This can be due to either open channel noise resulting from the activation of somatic nAChRs on the DA neurons by nicotine (Pidoplichko et al., 1997) or a dramatic reduction of the seal resistance due to bath application of nicotine. We never observed holding current changes exceeding 20 pA in the presence of nicotine, arguing against a loss of seal resistance due to nicotine bath application. The majority of the somatic nAChRs can be blocked by 5 μM mecamylamine (MEC) (Pidoplichko et al., 1997). In the presence of 5 μM MEC, the nicotine-induced increase in baseline noise was significantly reduced, and the noise level increased only by 10% ± 3% (Figure 3b; $p < 0.01$, n = 10). Thus, it is most likely that the increase in baseline noise results from the activation of somatic nAChRs by nicotine. Despite a doubling of the baseline noise, individual spontaneous EPSCs could still reliably be resolved in the presence of nicotine (Figure 3c; see also Experimental Procedures).

In contrast to increasing the frequency of spontaneous EPSCs (Figure 3d), nicotine did not alter the amplitude distribution of the spontaneous EPSCs (Figures 3e and 3f). This indicates that nicotine increases the probability of release by a presynaptic mechanism. The effect on spontaneous EPSCs was independent of action potential generation in presynaptic fibers, as the application of nicotine in the presence of tetrodotoxin (TTX) (2 μM) yielded similar results (data not shown). Thus, nicotine enhances excitatory synaptic transmission in the VTA through the activation of presynaptic nAChRs.

The effect of nicotine on the frequency of spontaneous EPSCs was concentration dependent (Figures 4a–4c). A nicotine concentration of 0.5 μM is reached in a smoker's blood immediately after smoking a cigarette, and this has been shown to have addictive effects (Corrigall and Coen, 1991; Henningfield et al., 1993; Corrigall et al., 1994; Pontieri et al., 1996). In our experiments, 0.5 μM nicotine increased the spontaneous EPSC frequency by nearly 2-fold (Figures 4b and 4c). The enhancement of spontaneous EPSC frequency was prevented in nine out of ten cells by MLA (10 nM), a specific antagonist of AChRs that contain the α7 subunit (Alkondon and Albuquerque, 1993; Seguela et al., 1993; McGehee and Role, 1995) (Figures 4d and 4f). In one of ten cells tested in the presence of MLA, nicotine increased the spontaneous EPSC frequency, suggesting the possibility that other nAChR subtypes may contribute to presynaptic enhancement in some cells. The non-α7 nAChR antagonist MEC (1 and 5 μM) inhibited the postsynaptic effect of nicotine in most VTA DA neurons but did not inhibit the effect of nicotine on the spontaneous EPSC frequency (Figures 4e and 4f; n = 12 of 14 cells). Together, these data indicate that the α7 subunit is an important component of the presynaptic nAChRs that mediate nicotine's effects on glutamate release in the VTA.

Recently, excitatory synapses on VTA DA neurons have been shown to express NMDA receptor–dependent long-term potentiation (LTP) following paired stimulation of the presynaptic inputs and the postsynaptic cell (Bonci and Malenka, 1999; Overton et al., 1999). In light of the pre- and postsynaptic expression of nAChRs at these synapses, we examined the effects of nicotine on LTP induction in these neurons. Figure 5a illustrates the LTP induction protocol, pairing presynaptic stimulation with transient postsynaptic depolarizations for 200 stimulations at a frequency of 1 Hz. Prior to LTP induction, control EPSCs were evoked by maximum-strength stimuli (see Figure 2b). Following the LTP stimulation paradigm, EPSC amplitude was increased to 122% ± 4% of control, and this increase persisted for at least 40 min (Figure 5b; n = 3). These findings are similar to previous reports of LTP induction in mesolimbic DA neurons (Bonci and Malenka, 1999).

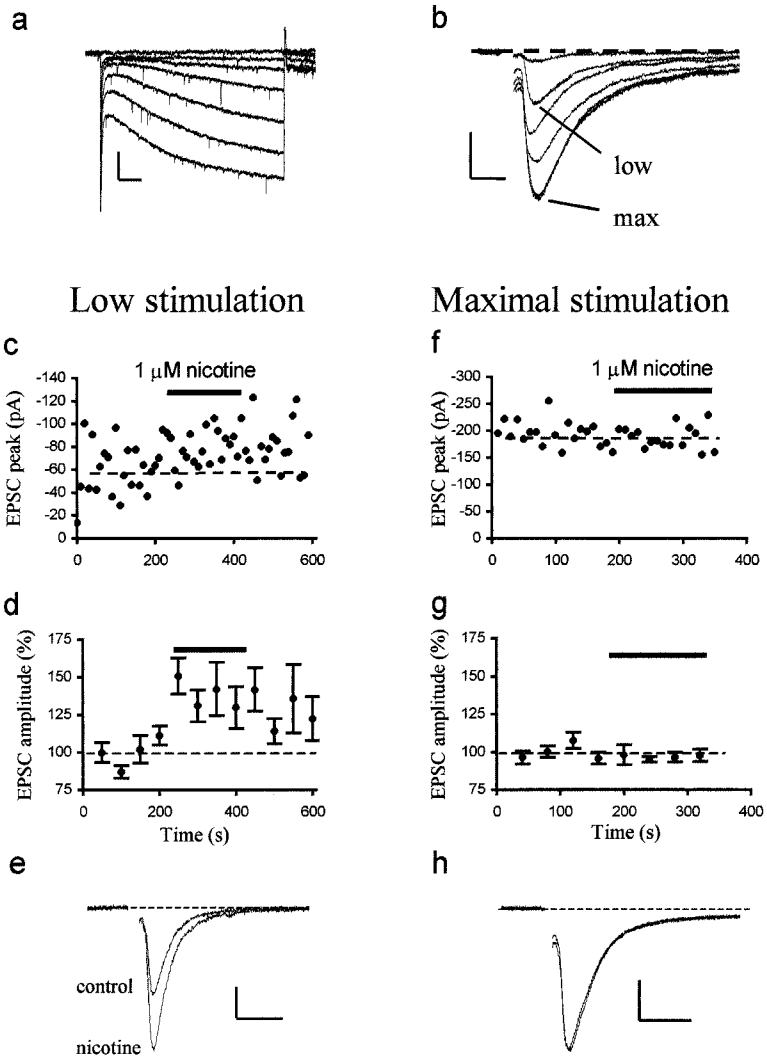

Figure 2. Nicotine Enhances Evoked Excitatory Synaptic Transmission to VTA DA Neurons

(a) Hyperpolarization-activated current, I_h, expressed in DA neurons. The membrane potential of a voltage-clamped VTA neuron was stepped from −60 mV to −120 mV in −10 mV steps.

(b) EPSCs evoked with varying stimulus strengths. Increasing the stimulus strength beyond that for the trace labeled "max" evoked EPSCs of the same amplitude, as shown by the overlapping traces evoked by different magnitude stimuli. Each trace is the average of ten EPSCs.

(c) Application of 1 μM nicotine (bar) increased the peak amplitude of the EPSCs evoked by low stimulation ($p < 0.05$). Each dot represents the peak amplitude of one EPSC.

(d) Data from eight cells were normalized and averaged to illustrate the response magnitude and time course for a population of cells. For each cell, five consecutive EPSCs were averaged and normalized to the control magnitude before nicotine application. The data were averaged for the eight of nine cells in which nicotine enhanced the EPSC amplitude significantly.

(e) Example of an EPSC evoked by low stimulation in the absence and presence of 1 μM nicotine. The stimulus artifact has been blanked.

(f) Nicotine does not change the amplitude of the EPSCs evoked by maximal stimulation.

(g) Average of seven experiments as in (f). Averages are calculated as in (d).

(h) Example of an EPSC evoked by maximal stimulation in the absence and presence of 1 μM nicotine.

Scale bars: (a) 50 pA, 250 ms; (b) 20 pA, 10 ms; (e) 25 pA, 10 ms; (h) 50 pA, 10 ms.

To examine whether the enhancement of glutamatergic synaptic transmission by nicotine can contribute to LTP induction, the pairing protocol was modified. Omitting the presynaptic stimulation, the postsynaptic depolarization was paired with a 200 s application of nicotine (1 μM). Figure 5c shows that the depolarization of the postsynaptic cell alone is not sufficient to induce the increase in synaptic strength (left bar). Pairing of nicotine application with the 200 depolarizations (right bar) increased the evoked EPSC to 116% ± 2.3% of control for at least 40 min (Figure 5c; n = 9 of 11 cells). Although smaller on average than the LTP induced by paired stimulation, the onset and duration indicate that this is a similar form of potentiation. To test whether LTP is induced by a presynaptic effect of nicotine on spontaneous glutamatergic transmission, we monitored the effect of nicotine on the spontaneous EPSC frequency during the modified pairing protocol. Also during LTP induction, nicotine increased the spontaneous EPSC frequency (Figure 5d). The amount of LTP induced by nicotine correlated well with the increase in EPSC frequency (Figure 5e; n = 3). The average increase in EPSC frequency plotted against the average amount of LTP induced by nicotine (closed dot in Figure 5e; n = 13 and 9, respectively) confirms the observed relation between EPSC increase and LTP induction.

To test whether the direct depolarization of the DA cell by nicotine (Pidoplichko et al., 1997) can contribute to LTP induction, we allowed the membrane potential of the postsynaptic cell to fluctuate using current clamp recording mode, while the presynaptic fibers were stimulated maximally (200 times at 1 Hz). Stimulating presynaptic fibers alone was not sufficient to induce LTP (Figure 6a, left bar; n = 5), and the pairing of stimulation with nicotine application was similarly ineffective (Figure 6a, right bar). Figure 6b shows that nicotine depolarization of the postsynaptic cell is sufficient to increase action potential frequency to 242% ± 87% (n = 5) of the control firing rate. Despite this excitatory effect, the stimulation of somatic nAChRs by nicotine does not contribute to LTP induction in the VTA.

In agreement with our finding that the enhancement of spontaneous EPSC frequency depends on α7-containing nAChR activation (Figure 4d), the LTP induced by 1 μM nicotine was inhibited by the presence of MLA (10 nM; Figure 7a; n = 5). The 1 μM nicotine-induced LTP was also prevented by the presence of 50 μM D-APV (Figure 7b; n = 5), showing that this effect requires activation of NMDA receptors. The effect of 1 μM nicotine on the frequency of spontaneous EPSCs was not affected by the presence of D-APV (Figure 7c). This shows that pairing-induced LTP and nicotine-induced

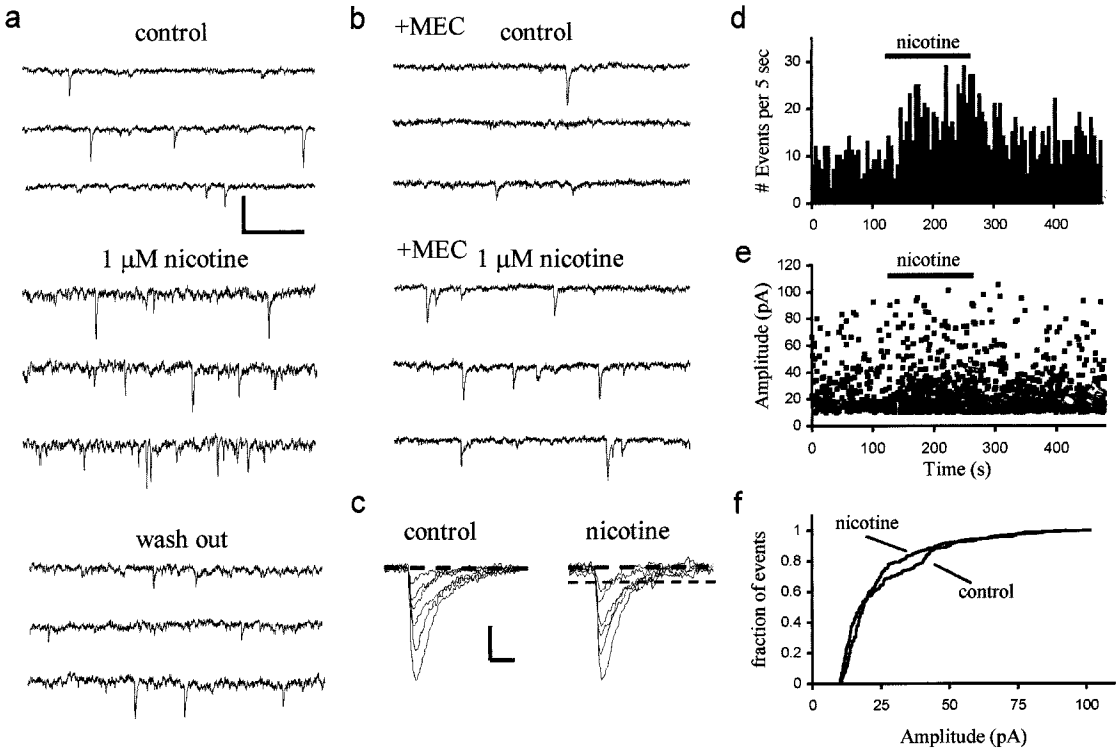

Figure 3. Nicotine Enhances Spontaneous EPSC Frequency in VTA DA Neurons

(a) Current traces showing spontaneous EPSCs. Scale bar: 50 pA, 100 ms.

(b) Similar experiment as in (a), but in the presence of 5 μM MEC, to illustrate that the increase in baseline noise by nicotine is prevented by MEC. Scale bar as in (a).

(c) Examples of individual spontaneous EPSCs, showing that despite the increase in baseline noise by nicotine, EPSCs can still be resolved reliably. The dotted line at the right shows the setting of the detection level in the analysis software (see Experimental Procedures). Scale bar: 20 pA, 5 ms.

(d) Frequency histogram of the same experiment as in (a) (bin = 5 s). Bar indicates the application of 1 μM nicotine.

(e) Amplitudes of individual spontaneous EPSCs of the experiment in (a) and (d). Each point represents the peak amplitude of a single EPSC.

(f) Cumulative plot of the amplitude distribution in (e), in the absence (control) and presence of 1 μM nicotine. The distributions showed no significant difference ($p = 0.12$, Kolmogorov-Smirnov test).

LTP share mechanistic elements, i.e., they both depend on the activation of NMDA receptors (Bonci and Malenka, 1999). At the same time, it shows that the long-term enhancement differs from the short-term enhancement described in Figures 2c–2e, since D-APV blocks the long-term effect but leaves the short-term increase of synaptic transmission unaffected (Figures 7b and 7c). Moreover, the EPSCs depicted in Figure 5c were evoked with maximum stimulus strength, on which nicotine had no direct short-term effect (Figures 2f–2h). Thus, we conclude that nicotine-induced enhancement of excitatory transmission in the VTA via presynaptic α7-containing nAChRs contributes to NMDA receptor–dependent LTP induction.

Discussion

Nicotine has been shown to induce prolonged increases in NAcc DA release in vivo, and this process is dependent upon NMDA receptor activation in the VTA (Imperato et al., 1986; Di Chiara and Imperato, 1988; Schilstrom et al., 1998a, 1998b). Our findings provide a mechanistic explanation at the cellular level for these in vivo observations. Although somatic nAChRs excite VTA DA neurons directly, this activation is transient due to subsequent receptor desensitization (Pidoplichko et al., 1997). We show here that activation of presynaptic α7-containing nAChRs on glutamatergic terminals can induce long-term potentiation of excitatory input to the VTA, which in turn leads to persistent increases in DA release in the NAcc independent of nAChR desensitization. Our findings show that nicotine alters synaptic function in the VTA using mechanisms that in other brain areas are thought to be responsible for learning and memory (Malenka and Nicoll, 1999).

The electrical activity of VTA DA neurons and the release of DA in the NAcc are strongly regulated by the activation of glutamate receptors in the VTA (Kalivas et al., 1989; Suaud-Chagny et al., 1992). Both NMDA and non-NMDA receptors are present on DA neurons in the VTA (Wang and French, 1993a), and they both increase firing rate (Suaud-Chagny et al., 1992; Wang and French, 1993a, 1993b). However, non-NMDA receptors increase sustained firing activity, whereas NMDA receptor stimulation induces burst firing (Charlety et al., 1991; Johnson et al., 1992; Suaud-Chagny et al., 1992; Chergui et al., 1993). Increases in both sustained and burst firing can enhance DA release in the NAcc, but burst firing is twice as efficient in augmenting DA release in the NAcc (Suaud-Chagny et al., 1992). Activation of the prefrontal

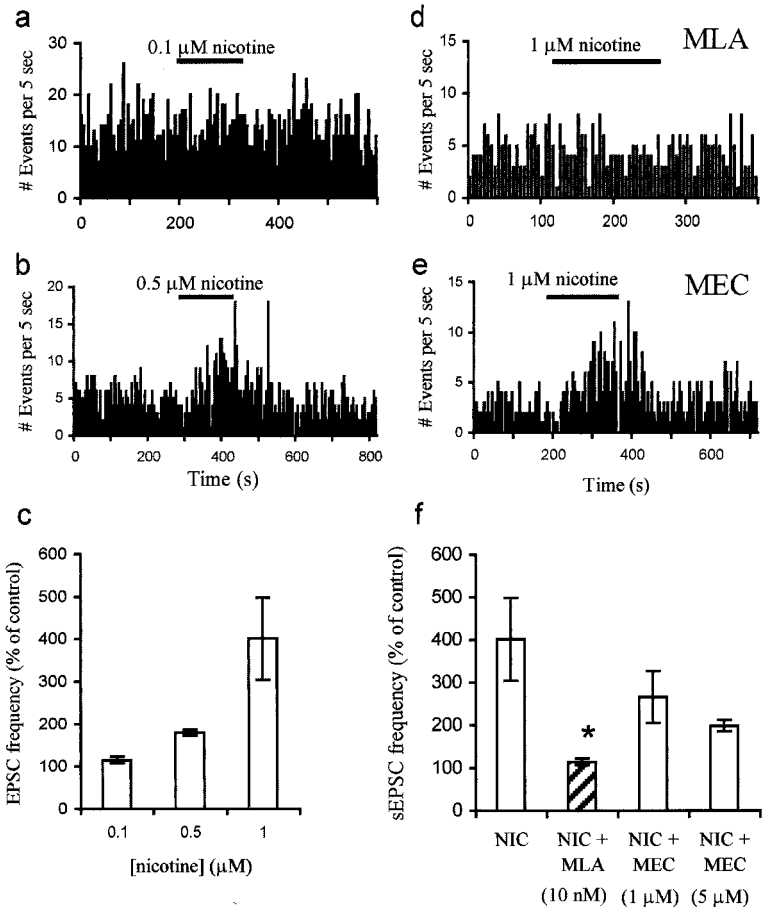

Figure 4. The Enhancement of Spontaneous EPSC Frequency Is Concentration Dependent and Is Mediated by nAChRs Containing the α7 Subunit

(a and b) Examples of the effect of 0.1 and 0.5 μM nicotine on the frequency of spontaneous EPSCs.

(c) Summary of the dependence of spontaneous EPSC frequency on nicotine concentration. Concentrations: 0.1 μM, n = 10; 0.5 μM, n = 11; 1 μM, n = 13.

(d) Pretreatment of the slice with MLA (10 nM, >15 min prior to nicotine) prevents the increase by the application of 1 μM nicotine (indicated by the bar).

(e) MEC (1 μM) pretreatment did not block the increase of spontaneous EPSC frequency induced by nicotine.

(f) Summary of the increase of spontaneous EPSC frequency by nicotine. The EPSC frequency in the presence of nicotine and MLA was significantly lower than when nicotine was present alone or with MEC ($p < 0.05$). The EPSC frequency with nicotine and with nicotine and MEC (1 μM) did not differ significantly. Nicotine, n = 13; MLA, n = 10; MEC, 1 μM, n = 4; MEC, 5 μM, n = 10.

cortex, which provides the main glutamatergic projections to the VTA, induces burst firing of VTA DA neurons and increases DA release in the NAcc (Sesack and Pickel, 1992; Murase et al., 1993; Taber et al., 1995). As we have shown, the enhancement of this glutamatergic input by the presynaptic nAChRs increases both NMDA and non-NMDA receptor stimulation. In vivo, the nicotine-induced enhancement of DA release in the NAcc can be diminished by the infusion of NMDA antagonists in the VTA (Schilstrom et al., 1998a). Thus, the presynaptic enhancement and the subsequent LTP of the glutamatergic synaptic inputs from prefrontal cortex to VTA that we describe here are likely mechanisms underlying these in vivo observations. Enhancement of the glutamatergic inputs to DA neurons certainly leads to increased DA release in the NAcc and contributes to the addictive effects of nicotine.

Presynaptic nAChRs containing the α7 subunit have been implicated to play an important role in neuronal plasticity throughout the CNS (McGehee and Role, 1995; Broide and Leslie, 1999). In brain areas such as the medial habenula, hippocampus, olfactory bulb, and sensory neocortex the release of glutamate is enhanced by α7 nAChR activation (McGehee et al., 1995; Alkondon et al., 1996; Gray et al., 1996; Aramakis and Metherate, 1998). Interestingly, in the hippocampus nicotine can facilitate the induction of LTP, but the type of nAChR involved is not known, nor whether the nAChRs are located pre- or postsynaptically (Fujii et al., 1999). A recent hypothesis is that α7 nAChRs and NMDA receptors have complementary roles in synaptic plasticity (Albuquerque et al., 1995; Broide and Leslie, 1999). Both α7 nAChRs and NMDA receptors have a high Ca^{2+} permeability, and activation of these receptors modulates intracellular Ca^{2+} concentrations. However, α7 nAChRs can conduct Ca^{2+} at resting membrane potentials, whereas NMDA receptors cannot, due to the Mg^{2+} blockade. Our data show that in the VTA α7 nAChRs and NMDA receptors can fulfill complementary roles in inducing a long-term enhancement of excitation of the brain reward system. Upon the arrival of nicotine in the VTA, activation of presynaptic α7 nAChRs allows the entry of Ca^{2+} in the glutamatergic terminals, which enhances glutamate release. This enhancement of glutamate release results in an increased activation of NMDA, as well as non-NMDA receptors, which leads to Ca^{2+} influx into the postsynaptic DA neuron and the induction of LTP. The short-lasting direct depolarization of VTA DA neurons by postsynaptic nAChRs (Pidoplichko et al., 1997; Picciotto et al., 1998) will facilitate the relief of Mg^{2+} blockade of the NMDA receptors and thereby contribute to the induction of LTP. This results in a long-term excitation of the brain reward system.

Although the contribution of this phenomenon to behavioral reinforcement awaits behavioral testing, these findings provide mechanistic insight into the dependence of nicotine-induced increase in DA release on both NMDA receptor and α7 nAChR activation in vivo

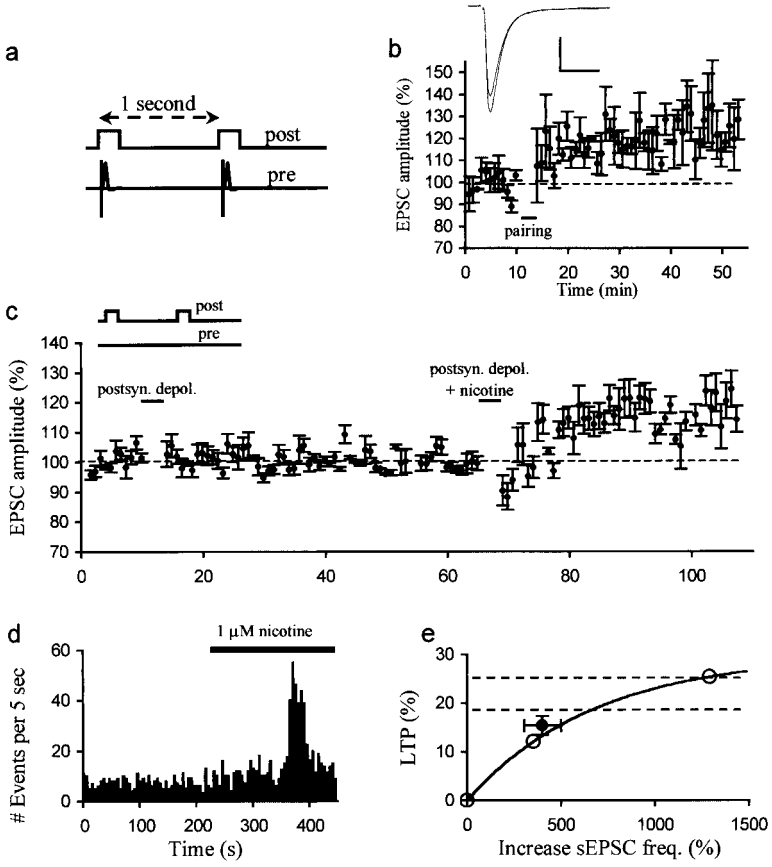

Figure 5. Presynaptic Stimulation by Nicotine and the Induction of LTP in VTA DA Neurons

(a) Diagram of the pairing protocol used to induce LTP. The postsynaptic cell was depolarized from −70 mV to +10 mV for 100 ms 200 times at 1 Hz. Simultaneously, the presynaptic inputs were stimulated maximally.

(b) Time course of the long-term increase of the evoked EPSC amplitude induced by the pairing protocol (indicated by bar). The normalized EPSC amplitude was determined as in Figure 2d. The inset shows example traces of evoked EPSCs before and after the pairing protocol. Scale bar: 50 pA, 25 ms.

(c) Nicotine increases the amplitude of the evoked EPSC when the postsynaptic cell is depolarized as in the pairing protocol (a), without stimulating the presynaptic fibers (n = 5). Bars indicate the application of the postsynaptic depolarizations. Nicotine was only present for the duration of the second series of postsynaptic depolarizations (200 s). The inset schematically shows the modified pairing protocol.

(d) During LTP induction nicotine enhances the frequency of spontaneous EPSCs.

(e) The increase in EPSC frequency induced by nicotine (1 μM) corresponds with the amount of LTP that is induced by nicotine (open dots, n = 3). The closed circle represents the average increase in EPSC frequency by 1 μM nicotine (n = 13) plotted against the average amount of LTP induced by 1 μM nicotine (n = 9). The dotted lines represent the range of LTP that is induced by normal pairing of pre- and postsynaptic activity.

(Schilstrom et al., 1998a, 1998b). Recently, mice lacking the β2 subunit were reported to display deficits in nicotine-induced DA release in the NAcc (Picciotto et al., 1998). In the same study, β2 −/− mice did not self-administer nicotine, providing compelling evidence of the importance of the β2 subunit in the CNS effects of nicotine. There is considerable variation both within and between species in the extent to which different animals will self-administer nicotine. Unlike rats that readily self-administer nicotine, the wild-type mice used in the study

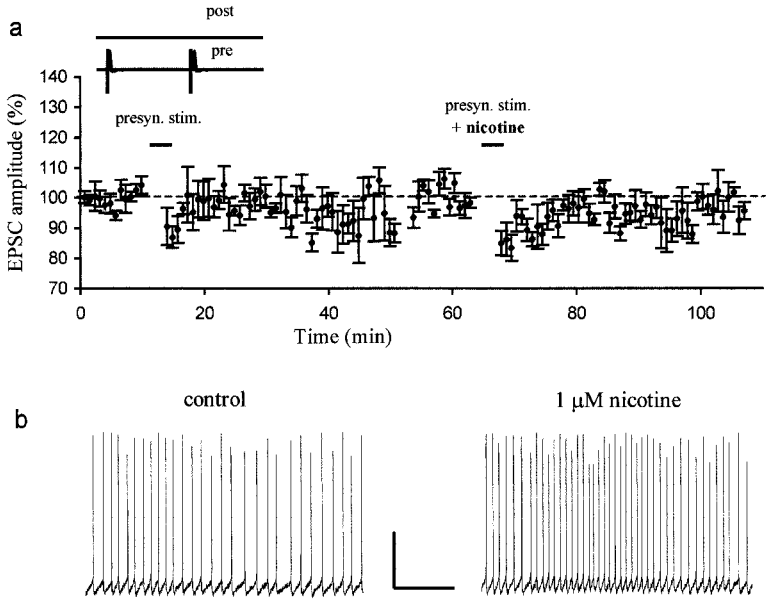

Figure 6. Postsynaptic Depolarization by Nicotine Does Not Contribute to LTP Induction

(a) Nicotine does not enhance EPSC amplitude when the protocol includes only presynaptic stimulation (n = 5). The inset shows how the pairing protocol was modified in this experiment. During the 200 stimulations of the presynaptic fibers (bars), the postsynaptic cell was held in current clamp, allowing the membrane potential to fluctuate. Nicotine was only present during the second series of presynaptic stimulations (200 s).

(b) VTA DA neurons fire action potentials spontaneously, and nicotine (1 μM) increases action potential frequency. Scale bar: 40 mV, 2 s.

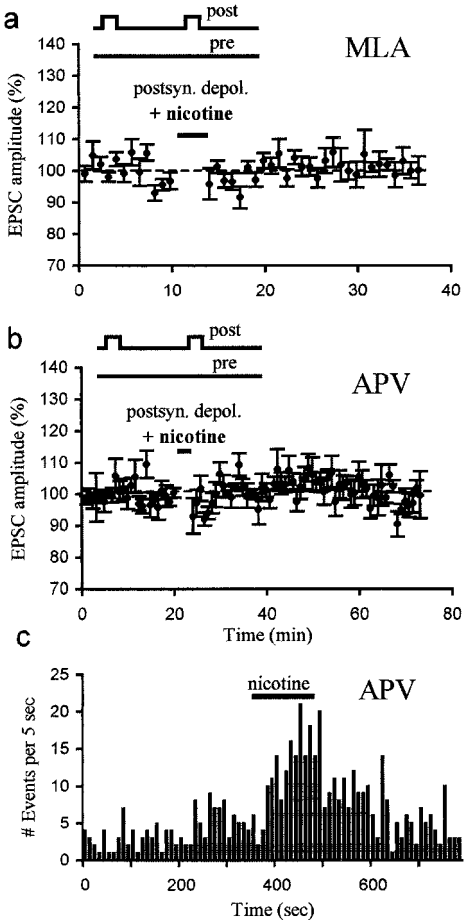

Figure 7. α7-Containing nAChRs and NMDA Receptors Contribute to the Induction of LTP by Nicotine

(a) MLA (10 nM) inhibited the long-term increase of the evoked EPSC amplitude induced by nicotine (1 μM, n = 5).

(b) Similar results were seen when the slices were pretreated with D-APV (50 μM, n = 5). Insets in (a) and (b) show the adapted pairing protocol. Bars in (a) and (b) indicate the presence of the postsynaptic depolarizations, as well as the application of 1 μM nicotine.

(c) Effect of 1 μM nicotine on the spontaneous EPSC frequency in the presence of D-APV (50 μM).

by Picciotto et al. required training with cocaine self-administration prior to testing nicotine infusion. In spite of the fact that nicotine causes increases in NAcc DA release in these mice, this is evidently not sufficient for initiation of nicotine self-administration in these animals. Thus, it is possible that these animals express lower levels of α7 on the glutamatergic terminals in the VTA, explaining the prominent dependence of these phenomena on β2-containing nAChRs. Although there is clear evidence that α7 can exist as a homomeric pentamer in the CNS (Drisdel and Green, 2000), there are also studies supporting heteromeric complexes that include α7 (Yu and Role, 1998). Thus, another possibility is that the β2 subunit combines heteromerically with α7 to influence DA release and behavior and the β2 mutants are lacking this receptor complex. Nicotine self-administration is clearly a complex phenomenon, dependent upon a myriad of physiological effects, and further studies are required to unravel this complexity.

Our data show that a brief nicotine application can induce LTP of the excitatory input to brain reward centers. This suggest that in humans a short nicotine exposure of a few minutes, even if there is no history of smoking, can cause long-lasting changes in excitatory transmission to the mesoaccumbens DA neurons. Given the correlation between NAcc DA release and behavioral reinforcement (Stolerman et al., 1995), this may be the important early step in the process of addiction. Other support for this idea has come from recent reports showing that a single exposure to amphetamines can cause long-term changes in both behavior and neurochemistry (Vanderschuren et al., 1999). Together, these findings suggest that the very first exposure to an addictive substance can leave its mark in the brain for a long time.

Experimental Procedures

Horizontal brain slices were prepared from Sprague-Dawley rats (10–20 days). Following isoflurane anesthesia, animals were decapitated and the brain was removed. After removal of the olfactory bulbs, the midbrain was cut at the level of the fourth ventricle and the brain was placed in ice cold artificial CSF solution (in mM: NaCl 125, KCl 2.5, $MgCl_2$ 1, $CaCl_2$ 2.5, glucose 20, NaH_2PO_4 1, $NaHCO_3$ 25, ascorbic acid 1; bubbled continuously with 95% O_2/5% CO_2). Two or three slices (250–300 μm) were cut in the cold solution and were placed in a holding chamber (32°C–34°C) to recover for at least 1 hr. For recording the slice was tranfered to a chamber superfused (~2 ml/min) with bicarbonate buffered solution without ascorbic acid, at room temperature.

Neurons were visualized under infrared illumination using an upright microscope (Axioskop, Zeiss). Electrodes (2–4 MΩ) contained (in mM): K-gluconate 154, KCl 1, EGTA 1, HEPES 10, glucose 10, and ATP 5 (pH 7.4 with KOH). Standard whole-cell voltage clamp recordings were made using an Axopatch 200B amplifier, a Digidata 1200 interface, and pCLAMP 7 (Axon Instruments). For evoked transmission, the current was filtered at 5 kHz and digitized at 20 kHz. Spontaneous transmission, hyperpolarization-activated currents (I_h) (Johnson and North, 1992), and voltage recordings were filtered at 1 kHz and digitized at 5 kHz. For perforated patch recordings (Horn and Marty, 1988; Rae et al., 1991) used in the experiments described in Figures 5–7, amphoterocin B (660 μg/ml final concentration, Sigma) dissolved in DMSO was added to the pipette solution (Bonci and Malenka, 1999). Experiments were started when the series resistance dropped below 40 MΩ, but was typically 15–30 MΩ. Series resistance in normal whole-cell recording was 4–8 MΩ. Neurons were held at −60 mV to assess the presence of I_h but were held at −70 mV throughout the rest of the voltage clamp experiments. A bipolar tungsten electrode (FHC) was placed 150–250 μm rostral of the recording site to evoke synaptic transmission at 0.1 Hz. To isolate excitatory glutamatergic transmission, experiments were done in the presence of 20 μM bicuculline (Sigma). The stimulus strength for the low, submaximal stimulation (Figures 2c–2e) was chosen such that EPSCs were evoked reliably without failures. The amplitude of evoked EPSCs was determined in real time using pCLAMP software. Student's t test was used to compare evoked EPSC amplitude before and after drug administration. Data are presented as means ± standard error.

Nicotine, MEC (both from Sigma), MLA (RBI), D-APV (Tocris), and TTX (Alomone Labs) were applied through bath perfusion. MLA, MEC, D-APV, and TTX were present in the bath at least 15 min before the effect of nicotine was assessed. A new slice was used for each experiment, so that neurons were exposed only once to nicotine. The only exceptions to this were tests of D-APV effects on the enhancement of evoked and spontaneous EPSCs in the same cells, and the nicotine applications were separated by >1 hr (Figures 7b and 7c).

Spontaneous EPSCs were analyzed offline using the Mini Analysis Program (Synaptosoft) and were selected using amplitude, rise time, and surface area criteria. As mentioned in the Results, nicotine doubled baseline noise due to the activation of somatic nAChRs. Therefore, the amplitude detection level for a given experiment was set to five times the standard deviation of the noise in the presence

of nicotine. In the majority of the experiments the amplitude detection level was set between 10 and 15 pA. Moreover, after the software selected individual spontaneous EPSCs, each detected event was checked separately by visual inspection to prevent noise from compromising the analysis. Student's t test was used to compare the average spontaneous EPSC frequency before and after drug administration. Statistical comparison of the cumulative amplitude distributions was carried out with the Kolmogorov-Smirnov test.

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