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Action-Potential-Independent GABAergic Tone Mediated by Nicotinic Stimulation of Immature Striatal Miniature Synaptic Transmission

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Liu Z, Otsu Y, Vasuta C, Nawa H, Murphy TH. Action-potential-independent GABAergic tone mediated by nicotinic stimulation of immature striatal miniature synaptic transmission. *J Neurophysiol* 98: 581–593, 2007. First published June 6, 2007; doi:10.1152/jn.00768.2006.—Stimulation of presynaptic nicotinic acetylcholine receptors (nAChRs) increases the frequency of miniature excitatory synaptic activity (mEPSCs) to a point where they can promote cell firing in hippocampal CA3 neurons. We have evaluated whether nicotine regulation of miniature synaptic activity can be extended to inhibitory transmission onto striatal medium spiny projection neurons (MSNs) in acute brain slices. Bath application of micromolar nicotine typically induced 12-fold increases in the frequency of miniature inhibitory synaptic currents (mIPSCs). Little effect was observed on the amplitude of mIPSCs or mEPSCs under these conditions. Nicotine stimulation of mIPSCs was dependent on entry of extracellular calcium because removal of calcium from perfusate was able to block its action. To assess the potential physiological significance of the nicotine-stimulated increase in mIPSC frequency, we also examined the nicotine effect on evoked IPSCs (eIPSCs). eIPSCs were markedly attenuated by nicotine. This effect could be attributed to two potential mechanisms: transmitter depletion due to extremely high mIPSC rates and/or a reduction in presynaptic excitability associated with nicotinic depolarization. Treatment with low concentrations of K^+ was able to in part mimic nicotine's stimulatory effect on mIPSCs and inhibitory effect on eIPSCs. Current-clamp recordings confirmed a direct depolarizing action of nicotine that could dampen eIPSC activity leading to a switch to striatal inhibitory synaptic transmission mediated by tonic mIPSCs.

INTRODUCTION

Miniature transmitter release results from the constitutive low-level release of individual vesicles of neurotransmitter. Since the 1950s this form of synaptic transmission was thought to be a reflection of a leaky evoked release mechanism, and it was not clear whether it had a function of its own (Otsu and Murphy 2003). Previous studies suggest that miniature release (mini) can reflect both the local chemistry of synapses (Murphy et al. 1994) as well as the network properties of neurons (Carter and Regehr 2002; Sharma and Vijayaraghavan 2003). Although mini rates are typically low, recent data describe how nicotine stimulation of nicotinic acetylcholine receptors (nAChRs) can elevate the frequency of excitatory glutamatergic minis to levels that can affect network behavior (Sharma and Vijayaraghavan 2003).

Acetylcholine (ACh) differs from other neuromodulators such as dopamine (DA), noradrenaline, and metabotropic glutamate receptor agonists in that it can affect presynaptic calcium levels through direct calcium influx via nAChRs and/or activation of voltage-sensitive calcium channels (VSCCs) resulting from depolarization induced by Na^+ influx through nAChRs (Dani 2001; Gray et al. 1996). This nAChR-mediated increase in presynaptic calcium is then associated with the increase in minis (Gray et al. 1996; Guo et al. 1998; Kiyosawa et al. 2001; Lena and Changeux 1997; Sharma and Vijayaraghavan 2003). In striatum, multiple nAChR subunits are expressed (Champtiaux et al. 2003; Wada et al. 1989; Zoli et al. 2002), and evidence suggests that presynaptic nAChRs regulate the secretion of DA from substantia nigra DAergic terminals (Champtiaux et al. 2003; Marshall et al. 1997; Zhou et al. 2001). However, relatively little is known about the effect of nicotine on the release of fast transmitters such as glutamate and GABA in the striatum (Kita 1996; Koos and Tepper 2002; Misgeld et al. 1980).

About 90% of total striatal neurons are medium spiny neurons (MSNs) that employ GABA as a neurotransmitter and project to other regions such as substantia nigra pars reticulata and globus pallidus. MSNs receive inhibitory and excitatory afferents predominantly from GABAergic interneurons and from the cerebral cortex and thalamus, respectively (for review, see Wilson 2004). Connections between MSNs are rare (Koos et al. 2004), especially in mature striatum, although recently more direct evidence with dual patch-clamp recording have demonstrated connections between MSNs in ventral striatum of relatively mature brains (Taverna et al. 2004). It is possible that GABA- or glutamatergic transmission onto MSNs is presynaptically modulated by ACh released from cholinergic interneurons. Given the existence of nAChRs on presynaptic terminals that innervate MSNs and the nAChRs' unique ability to increase presynaptic calcium concentration, we determined whether miniature synaptic activity in the striatum might be regulated by this mechanism. We report that nicotine can produce almost 12-fold increases in striatal GABAergic miniature inhibitory synaptic currents (mIPSCs) but not glutamatergic miniature excitatory postsynaptic currents (mEPSCs) through a mechanism associated with calcium entry through calcium-permeable nicotinic receptors. These high rates of minis stimulated by nicotine dampen evoked

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IPSCs (eIPSCs), suggesting a transition to a mode of signaling involving tonic mIPSC-mediated inhibition.

METHODS

Slice preparation

Wistar rats (postnatal day 8–15) were anesthetized with halothane and decapitated. All animals used in this project were cared for in accordance with regulations of the Canadian Council on Animal Care. Coronal slices of striatum (250–300 μm thickness) were prepared in an ice-cold modified artificial cerebrospinal fluid (ACSF) and incubated at room temperature for ≥1h in normal ACSF. The composition of normal ACSF was as follows (in mM): 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, and 11 glucose (pH 7.4 with 95% O_2-5% CO_2). In the modified ACSF, NaCl was substituted for 200 mM sucrose, and the solution contained 0.8 mM $CaCl_2$, 4 mM $MgSO_4$, and 1 mM kynurenate.

Electrophysiology

MSNs in striatum were visualized using a water-immersion objective lens (Olympus ×60) and were identified by shape and size (ovoid cell body with 8–14 μm major axis). A whole cell patch electrode (~4 MΩ) was used to record synaptic responses from these neurons in a voltage- or current-clamp mode with an Axopatch 200B. Series resistance was compensated 60–70%. The cells were clamped at 0 or −65 mV to record inhibitory synaptic currents or excitatory synaptic currents, respectively. To record mIPSCs, the recording solution included 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM), D,L-2-amino-5-phosphonovaleric acid (APV; 100 μM) and tetrodotoxin (TTX; 1 μM). Spontaneous and evoked IPSCs (sIPSCs and eIPSCs) were recorded in the absence of TTX. A bipolar tungsten electrode was put in the striatum (~200 μm from a recording cell) to induce eIPSCs. Current pulses were delivered through the electrode for 150 μs at a range from 100 to 500 μA (1.1–1.8 times higher than threshold intensity). To record mEPSCs, bicuculline methiodide (BMI; 20 μM) and 1 μM TTX were added to the recording solution. For voltage-clamp recording, the routinely used the internal solution contained (in mM): 123 Cs-methane sulfonate, 10 HEPES, 7.5 CsCl, 0.2 EGTA, 8 NaCl, 4 MgATP, 0.3 Na_2GTP, 5 QX314-Cl, and 5 Biocytin-Cl. In one set of experiments where constant negative holding potentials were used to assess mIPSCs as inward currents, we used a modified internal solution of the following composition (in mM): 130 CsCl, 10 HEPES, 0.2 EGTA, 4 MgATP, 0.3 Na_2GTP, 5 QX314-Cl, and 5 Biocytin-Cl. The pH of the two internal solutions was adjusted to 7.2 by CsOH and the osmolarity was adjusted to 290 mosM. The liquid junction potential was not corrected. When responses were monitored with current-clamp mode, the composition of internal solution was changed as follows (mM): 129.4 K-gluconate, 10 HEPES, 11.1 KCl, 0.02 EGTA, 4 NaCl, 3 MgATP, and 0.3 Na_2GTP (pH adjusted to 7.25 using KOH). To set the equilibrium potential for chloride to –58 mV as observed in MSNs (Jiang and North 1991), the recording bath solution was changed as follows (mM): 125 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.5 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 9 glucose (pH 7.4 with 95% O_2-5% CO_2) (Koos and Tepper 1999). Recordings were all performed at room temperature. Nicotine was applied with a local perfusion system equipped with a Y-shape tube or bath application. Various drugs to modify effects of nicotine were applied ≥10 min before agonist treatment. In the case of bath application, it takes ~2 min to reach drug solution into the recording chamber from its reservoir. In figures, *time 0* indicates the time at which the reservoir was changed, not the time of bath equilibration. Signals were digitized at 5 kHz and filtered at 2 kHz (low-pass Bessel filter). Drugs used were obtained from the following sources: TTX, nicotine tertrate, APV, BMI, mecamylamine (MEC), methyllycaconitine (MLA), and SKF38393 from Sigma; CNQX from Tocris; QX-314-Cl from Alomone labs; (+) SCH23390 and (−) sulpiride from RBI.

Histology

After recording, some slices were randomly chosen for biocytin-staining to confirm that the recorded cells possess the characteristic morphology of MSNs. Slices with biocytin-injected cells were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (PB) for >2 h at 4°C and soaked in 30% sucrose in phosphate-buffered saline (PBS) for another 12 h. The tissue was frozen with dry ice and thawed twice and then incubated for 30 min in PBS containing 0.5% H_2O_2 and 10% ethanol at room temperature (r.t.) to suppress endogenous peroxidase activity. The tissues were rinsed in PBS for 1 h and incubated in PBS containing 1% Triton X-100 (TX) for 4 h at r.t. After washing for 1 h, they were incubated in a 0.5% TX in PBS containing avidin-biotin-peroxidase complex (ABC solution; ABC Elite, Vector Laboratories) for 3 h at r.t. Visualization of biocytin-injected cells was achieved with 0.05M Tris-HCl buffer (pH 7.4) containing 3,3′-diaminobenzidine tetrahydrochloride (DAB; 0.05%) and 0.01% H_2O_2.

Data analysis

Spontaneous and miniature synaptic currents data were analyzed with AxoGraph 4 and pClamp 9 (Axon Instruments). An event was detected with a criterion of a threshold >3×SD of baseline noise and >3- to 5-pA amplitude. The detected mIPSCs were then manually inspected to exclude false events caused by an artificial source such as environmental noise. In young neurons, the frequency of mIPSCs could be very low leading to massive fold changes in mIPSC frequency (>100-fold was observed at times) after addition of nicotine. These very large fold changes in frequency made it difficult to assess the pharmacology of nicotine and its mechanism. Therefore only cells that had a basal frequency of mIPSCs between 0.1 and 2 Hz were used to assess the pharmacology and mechanism of nicotine effects. To evaluate the change of GABAergic activity, the percentage increase in mIPSC frequency was routinely used and was determined by comparing the average number of events per bin (1 bin = 20 s) during the last 2 min of the control period with the average during the peak of the nicotine effect in which 3 bins (before, peak, and after) were averaged. Data from cells with unstable baseline firing (≥15% fluctuation during the last 3 min of the control period) are not included. The average values are provided as means ± SE. The differences in the mean peak frequency were tested by Mann-Whitney *U* test.

RESULTS

Effect of nicotine on membrane excitability in MSNs

The effect of nicotine on MSNs in striatal slices from 8- to 15-day rat pups was studied to determine whether nAChR stimulation affects their excitability. MSNs were identified by their unique electrophysiological and morphological characteristics. MSNs had a relatively hyperpolarized resting potential (−76.3 ± 1.4 mV, $n = 11$), inward rectification (Fig. 1*A, a* and *b*), and spiny dendrites (Fig. 1*Ac*). These properties are consistent with previous work (Kawaguchi 1992; Kawaguchi et al. 1989; Tepper et al. 1998). To assess potential effects of nicotine on MSNs, we performed recordings in current-clamp mode to determine whether nicotine led to changes in MSN excitability. For these experiments, we used intracellular Cl^- concentrations (15 mM), which correspond to a –58-mV reversal potential for chloride that is consistent with previously reported values from intracellular recordings (Jiang and North 1991; Koos and Tepper 1999). Addition of 20 μM nicotine via bath application under current clamp led to an increase in noise

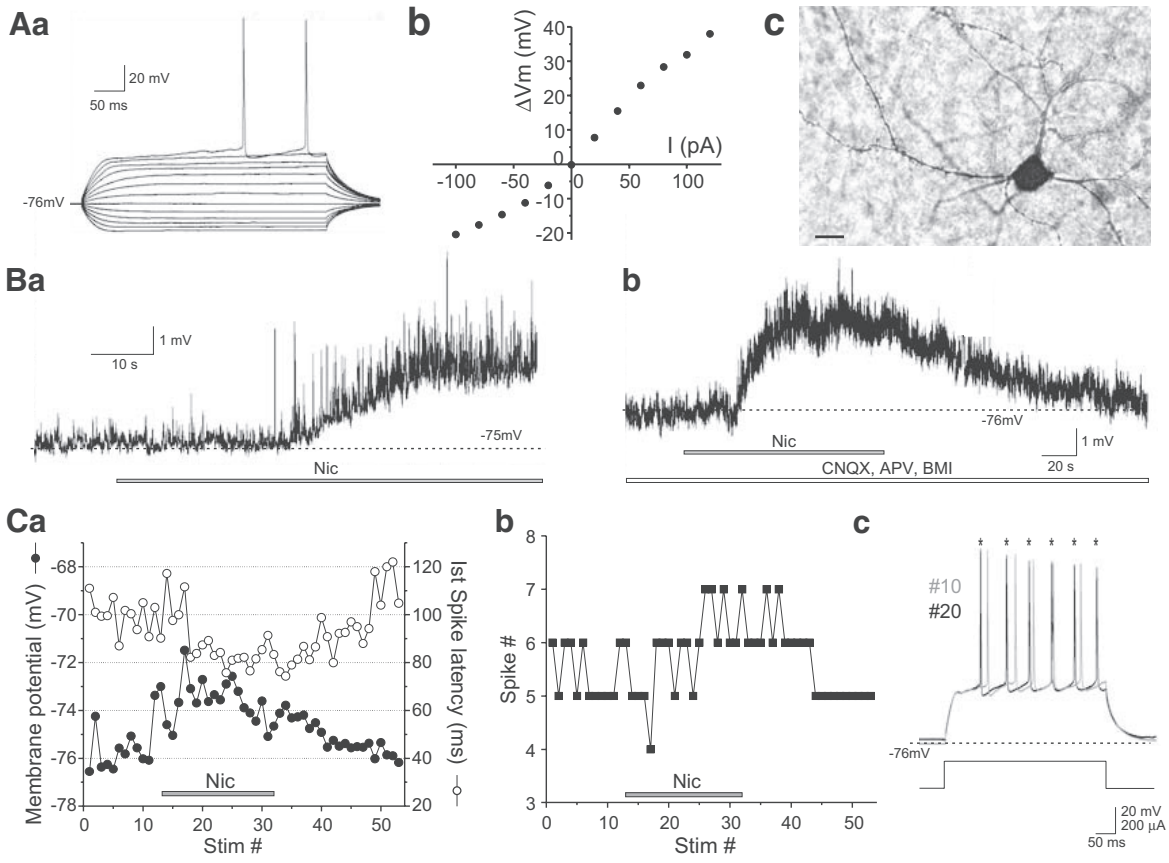

FIG. 1. Effect of nicotine on membrane potential and excitability in medium spiny neurons (MSNs). *A*: characteristic membrane properties and morphology of a MSN. *Aa*: traces show individual voltage responses to series of 400-ms current pulses from −100 pA with 20-pA increasing current steps. *Ab*: relationship between injected current and steady-state voltage responses are plotted. Note the inward rectification of MSNs. *Ac*: example of a MSN stained after recording. The scale is 10 μm. *B*: nicotine (20 μM) directly depolarizes MSNs in current-clamp mode. *Ba*: membrane depolarization and increased noise were observed during application of 20 μM nicotine under normal conditions. *Bb*: membrane depolarization was not blocked, but increased noise was decreased in the presence of glutamate [10 μM 6-cyano-7-nitroquinoxalene-2,3-dione (CNQX) and 100 μM 2-amino-5-phosphonovaleric acid (APV)], and GABA [20 μM bicuculline methiodide (BMI)] receptor blockers. Although 20 μM nicotine depolarized the neurons in current-clamp mode, it failed to produce action potentials as the firing threshold for MSNs was positive to –35 mV. *C*: minimal change in excitability after nicotine treatment. To assess changes in excitability, we applied depolarizing injections of current through the recording electrode during a control period or during the application of 20 μM nicotine. *Ca*: example of 20 μM nicotinic effect on membrane potential and spike latency. Nicotine depolarized the membrane potential by only a few millivolts, which was measured by averaging membrane potential for 3 s before current injection (filled circles) and shortened the 1st spike latency, which was the time between onset of current injection and the peak of the 1st action potential (white circles). Raw traces of stimuli 10 and 20 are shown in *Cc*. *Cb*: during nicotine application, a small increase in the number of spikes induced by each current injection was detected. *Cc*: 2 traces, before (10; gray) and during nicotine (20; black) application are superimposed. Asterisk, position of action potential in trace 20. Note the 1st spike latency is shortened and the resting membrane potential was depolarized during nicotine application.

and a modest depolarization of MSNs (3.17 ± 0.3 mV; $n = 3$; Fig. 1*Ba*), but action potentials (APs) were not induced. This increased noise was blocked in the presence of GABAergic (20 μM BMI) and glutamatergic (10 μM CNQX and 100 μM APV) receptor blockers. However, the modest depolarization persisted (Fig. 1*Bb*). The noise was thought to be induced by an increase in GABA or glutamate release, whereas the depolarization could be induced by a direct postsynaptic effect of nicotine on MSNs.

To assess whether nicotine altered spike threshold, MSNs were held at resting potential in current-clamp recording mode, and depolarizing pulses that induced three to five spikes with a first-spike latency of 100–120 ms were injected at 0.1 Hz. Bath application of 20 μM nicotine caused membrane depolarization and a shortened first-spike latency (113.3 ± 11.6 ms under control condition vs. 82.0 ± 3.3 ms in the presence of nicotine; $n = 3$; Fig. 1*C, a* and *c*) and a slight increase in spike number with threshold depolarizing pulses (4.1 ± 1.3 spikes under control condition vs. 5.0 ± 1.1 spikes in the presence of nicotine; $n = 3$ cells; Fig. 1*Cb*). In summary these experiments indicated a limited effect of nicotine on the excitability of MSNs.

Effect of nicotine on miniature synaptic events

In current-clamp experiments, we detected an inhibition of nicotine-induced noise by glutamatergic and GABAergic antagonists (Fig. 1*B, a* and *b*). To determine whether this effect was induced by presynaptic GABAergic or glutamatergic activity, we monitored miniature synaptic events under voltage-clamp recording mode. Miniature inhibitory postsynaptic currents (mIPSCs) were recorded as outward currents at a holding potential of 0 mV in the presence of CNQX (10 μM), APV (100 μM), and TTX (1 μM; Fig. 2*Aa*). mIPSCs were abolished by addition of BMI (20 μM), a $GABA_A$ receptor antagonist (data not shown). Thus the pharmacological data indicated that both Y-tube ($n = 8/8$) or bath ($n = 13/13$) application of 20 μM nicotine results in a robust increase in mIPSC frequency.

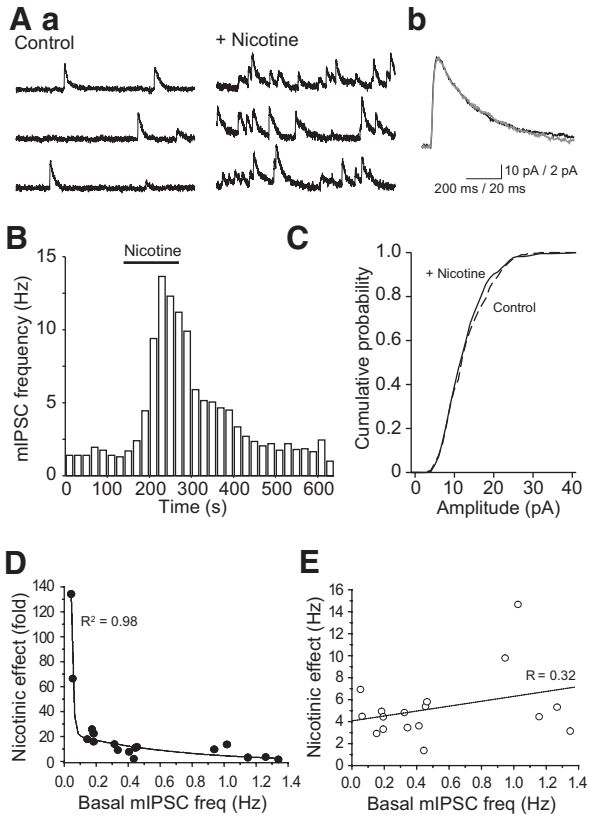

FIG. 2. Nicotine increases the frequency of mIPSCs in MSNs. *Aa*: whole cell voltage-clamp recording traces are shown depicting mIPSCs (in the presence of 1 μM TTX, 100 μM APV, and 10 μM CNQX) and with addition of 20 μM nicotine through the Y tube. Holding voltage was 0 mV. *Ab*: representative averaged traces (13 sweeps) showing mIPSC kinetics with (black trace) and without (gray trace) nicotine treatment. The 2 traces are overplotted and normalized to the peak current for control and nicotine cases. The decay τ was 24.7 ms. *B*: histogram showing increases in mIPSC frequency in a striatal neuron. In this cell, nicotine applied through Y-tube produces about a fivefold increase in mIPSC frequency that reverses within minutes of washout. *C*: cumulative probability histogram demonstrating mIPSC amplitude distribution under control conditions (303 events) and with 20 μM nicotine (440 events) from the same cell as in *B*. No significant difference in mIPSC amplitude was observed after nicotine treatment [Kolmogorov-Smirnov (KS) test; $P = 0.44$]. *D*: relationship between nicotinic enhancement and basal mIPSC frequency. Basal mIPSC frequency and the fold change in frequency after 20 μM nicotine treatment are plotted. Cells with relatively low basal mIPSC frequency have the largest fold increase during nicotine treatment ($r = -0.68$, $P = 0.01$). *E*: peak mIPSC frequency as a function of basal mIPSC frequency. Nicotine stimulates all cells to a similar frequency of mIPSCs regardless of their basal mIPSC frequency ($r = 0.096$, $P = 0.76$).

In Fig. 2*A*, representative traces are shown during control and after 1 min of 20 μM nicotine local application through Y-tube perfusion. Use of a semi-automated mIPSC detection system indicated an increase in mIPSC frequency > 10-fold (11.8 ± 1.9-fold, $n = 15$ cells) with 20 μM nicotine application (Fig. 2*B*). The effect of nicotine was fully reversible on washout and was concentration-dependent as shown in our data obtained with 1 and 100 μM nicotine (Fig. 8*Bb* and supplementary Fig. 1[1]).

In contrast to effects of noradrenaline in hypothalamus (Gordon and Bains 2005), analysis of mIPSC amplitudes demonstrated that nicotinic stimulation led to an increase only in frequency and not in amplitude [Fig. 2*C*, $n = 9/13$, $P < 0.01$; Kolmogorov-Smirnov (KS) test]. In a subset of neurons (4 cells), we did observe some apparent changes in amplitude. Due to the high frequency of events in some cases apparent increases in amplitude could be due to random summation of mIPSCs given their relatively long time course. In addition, the time course of mIPSCs was unaffected by nicotine treatment (Fig. 2*Ab*). Nicotinic agonist effects were detected in all MSNs examined, and cells with relatively low basal mIPSC frequency had the largest fold increase during nicotine treatment (Fig. 2*D*). To make our pharmacological analysis more quantity reliable, we did not include neurons with basal frequency < 0.1 Hz. However, there was no significant correlation between frequency in control and nicotine treatment (Fig. 2*E*).

Although 20 μM nicotine robustly elevated the frequency of mIPSCs, the effect was selective since analysis of mEPSCs recorded at −65 mV under pharmacological blockade of $GABA_A$ receptors (using 20 μM BMI) and 1 μM TTX indicated no significant effect of nicotine on the frequency of mEPSCs (Fig. 3, *Aa, B,* and *D*). In addition to there being no apparent effect on mEPSC frequency, we did not detect any change in the mEPSC kinetics (Fig. 3*Ab*) or amplitude (Fig. 3*C*, $n = 5/5$, $P > 0.1$; KS-test), indicating a specific action of nicotine on inhibitory presynaptic terminals.

Calcium dependency of nicotine effect on mIPSC frequency

To examine potential mechanisms of nicotine presynaptic action at GABAergic neurons, we determined whether the effect of nicotine on mIPSC frequency was calcium dependent. Calcium was removed from perfusing solution and substituted with additional Mg^{2+} (Ca^{2+}: 0 mM, Mg^{2+}: 3.8 mM) because the nicotinic receptor can be blocked by low level of divalent ions (Adams and Nutter 1992; Liu and Berg 1999). Under this condition, the baseline frequency of mIPSCs exhibited only a partial dependence on the extracellular calcium (0.93 ± 0.17 Hz in normal calcium ACSF compared with 0.50 ± 0.06 Hz in calcium-free ACSF, $P = 0.0084$, $n = 6$). In contrast, the effect of nicotine on mIPSC frequency was completely abolished ($P = 0.0003$, compared with that in normal calcium ACSF, $n = 8$; Fig. 4, *B* and *D*) by perfusion with calcium free ACSF. In analyzing results from this experiment, nicotine effects were assessed by comparison to the baseline mIPSC frequency of a 2-min period immediately before nicotine application when the slice was perfused with calcium free solution.

In a separate group of experiments, we determined whether nicotine's lack of an effect in 0 extracellular calcium could be reversed by perfusion with calcium containing ACSF in the continued presence of nicotine. As shown in supplementary Fig. 5, MSNs were still capable of responding to nicotine when extracellular calcium concentration was brought up to normal levels (fold increase = 11.7 ± 4.7, $P < 0.05$ comparison between basal frequency during a 2-min period before nicotine application in calcium-free ACSF and the peak frequency during nicotine application, $n = 3$). This result is not surprising since after an initial brief desensitization, there remains a significant amount of sustained nicotine conductance (Zhang et al. 1994). The result also further supports the proposal that extracellular calcium is required for nicotine to stimulate mIPSC frequency. Importantly, the increase in mIPSC frequency observed on re-addition of extracellular calcium was much greater than the effect of altered extracellular calcium on

[1] The online version of this article contains supplemental data.

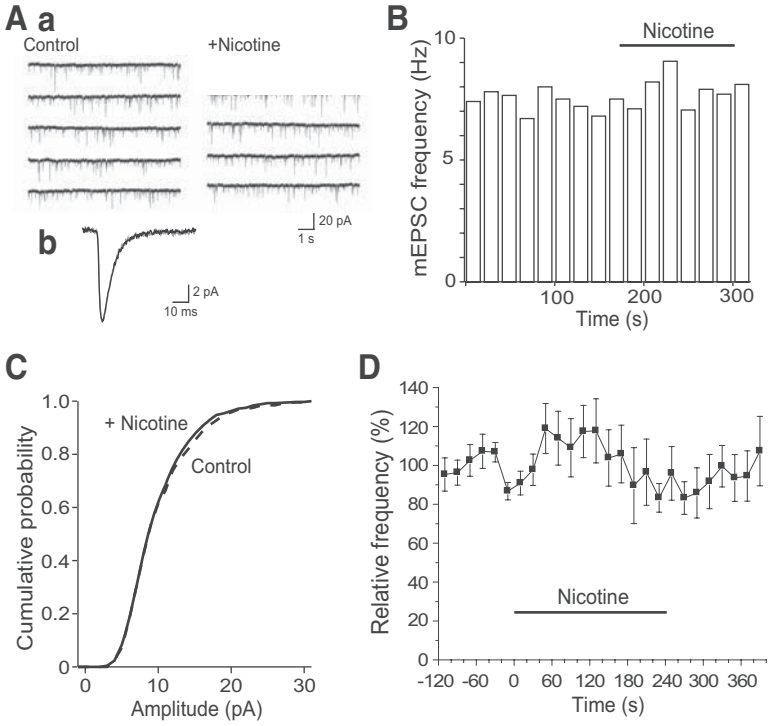

FIG. 3. No significant effect of nicotine on mEPSC frequency in MSNs. *Aa*: representative traces of mEPSCs recorded in the presence of 1 μM TTX and 20 μM BMI at −65-mV holding potential. In this example and group data, no significant change in frequency or amplitude of mEPSCs was observed with 20 μM nicotine treatment for 2 min through Y-tube. *Ab*: representative average mEPSCs (23 sweeps) from control conditions (gray trace) and after treatment with 20 μM nicotine (black trace). No significant difference in mEPSC kinetics was observed. *B*: time-course analysis of nicotine effect on mEPSC frequency. *C*: cumulative probability histogram demonstrating mIPSC amplitude distribution under control conditions (641 events) and with 20 μM nicotine (676 events), showing little effect of nicotine on mEPSC amplitude (KS-test; $P = 1.00$). *D*: group data of nicotine effect on mEPSC frequency ($n = 5–6$ cells). mEPSC frequency in each cell is normalized to an average frequency measured over 2 min before nicotine application. There was little effect of nicotine on mEPSC frequency.

basal mini frequency (1.93 ± 0.33-fold for change from normal calcium to calcium-free solution; 11.7 ± 4.7-fold change in mIPSC frequency from calcium-free solution in presence of nicotine to normal calcium solution with continued presence of nicotine; $P < 0.05$).

Because our current-clamp result indicated a postsynaptic depolarizing effect of nicotine in MSNs, we examined the possible involvement of VSCCs in the enhancement of mIPSC frequency. Presumably striatal intrinsic interneurons (or other GABAergic neurons) might also be depolarized by nicotine. To completely shut down VSCC activity, we used high concentrations of $CdCl_2$ and $NiCl_2$ as blocking agents. Cd^{2+} is known to block all VSCCs, whereas Ni^{2+} is more selective for low-threshold VSCCs (Herrington and Lingle 1992). $CdCl_2$ (200 μM) and $NiCl_2$ (1 mM) were washed in for 10 min before nicotine (20 μM) was applied in the presence of $CdCl_2$ and $NiCl_2$. The results showed that despite the presence of saturating VSCC blockers nicotine was still able to induce a robust increase in GABAergic mIPSC frequency (fold increase = 9.38 ± 1.8, $P = 0.60$, $n = 11$; Fig. 4, *C* and *D*).

Pharmacological characterization of presynaptic nAChR

The preceding results suggested that GABA release was enhanced after calcium influx through presynaptic nAChRs. In striatum, multiple nAChR subunits are expressed (Champtiaux et al. 2003; Wada et al. 1989; Zoli et al. 2002), including low levels of the α7 subunit (Dominguez del Toro et al. 1994; Seguela et al. 1993; Zoli et al. 2002). nAChR containing the α7 subunit are known to have a relatively high calcium permeability in neurons (Castro and Albuquerque 1995; Seguela et al. 1993; Vernino et al. 1994), and their resulting calcium influx may cause an enhancement of neurotransmitter release (Gray et al. 1996; McGehee et al. 1995; Vijayaraghavan et al.

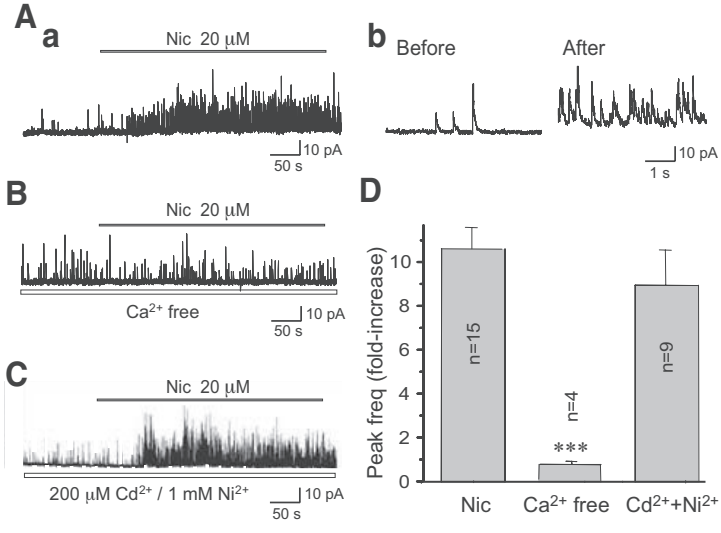

FIG. 4. Calcium-dependence of nicotinic stimulation of mIPSC frequency Current traces from a striatal neuron in the presence of 1 μM TTX, 100 μM APV, and 10 μM CNQX at 0-mV holding potential showing the effect of nicotine under various conditions. *Aa*: nicotine (20 μM) enhanced mIPSC frequency during bath application. *Ab*: 2 traces before and 100 s after nicotinic application from *Aa* are expanded. *B*: pretreatment with 0 mM extracellular calcium solution blocked the nicotine effect. *C*: nicotine enhancement of mIPSC frequency is independent of voltage-sensitive calcium channel (VSCC) activation. MSNs were treated with 20 μM nicotine in the presence or absence of a combination of $CdCl_2$ (200 μM) and $NiCl_2$ (1 mM). Despite the presence of saturating concentrations of VSCC blockers, nicotine still had a pronounced effect on mIPSC frequency. *D*: average increase in mIPSC frequency by 20 μM nicotine in the presence of normal extracellular calcium (Nic), pretreatment of 0 mM calcium in the extracellular solution (Ca^{2+} free) and pretreatment of 200 μM $CdCl_2$ and 1 mM $NiCl_2$. Comparisons among these 3 groups were made with Mann-Whitney (MW) test showing that in the absence of extracellular calcium, nicotine loses its ability to increase mIPSC frequency ($P = 0.0003$) while the cocktail of VSCCS blockers $CdCl_2$ (200 μM) and $NiCl_2$ (1 mM) fails to bring down the nicotine effect ($P = 0.60$).

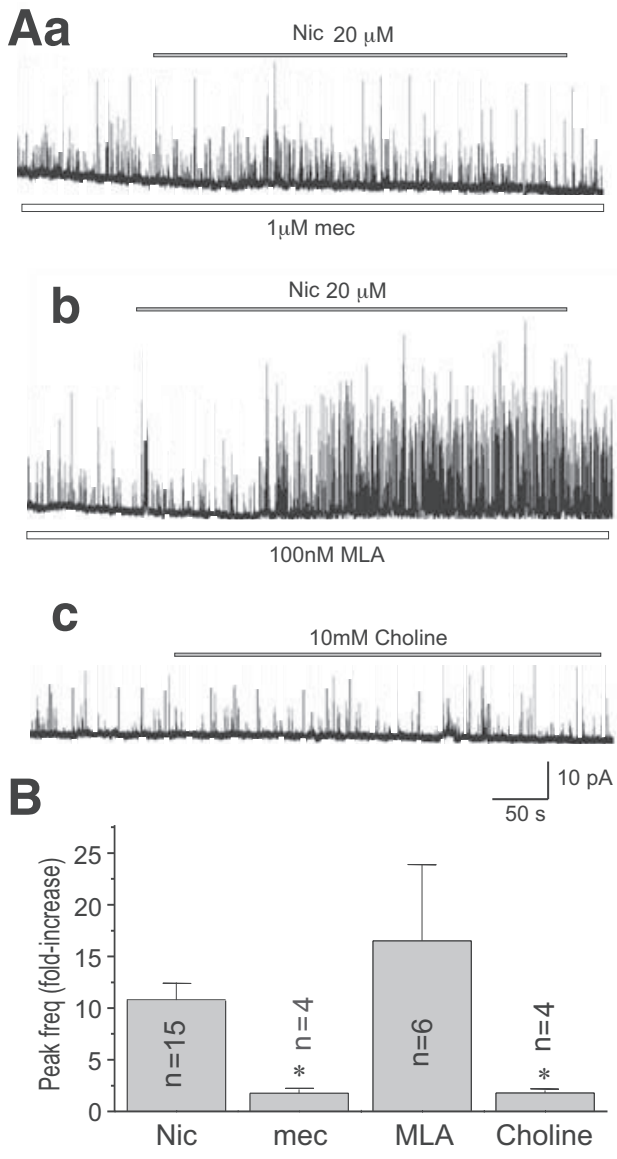

FIG. 5. Pharmacology of nicotinic enhancement of mIPSC frequency. *A*: representative traces showing nicotine effects on mIPSCs in the presence of 1 μM mecamylamine (*a*) and 100 nM methyllycaconitine (*b*). There was no effect of 10 mM choline application (*c*). *B*: average change in mIPSC frequency during the application of 20 μM nicotine (Nic) or 10 mM choline (choline), and nicotine added with the antagonists mecamylamine (MEC) or methyllycaconitine (MLA). Note: in both bars, the number of cells tested is indicated. Statistical significance was assessed using the MW test: nicotine ($n = 15$) vs. MEC ($n = 4$, $P = 0.0191$), MLA ($n = 6$, $P = 0.40$), and choline ($n = 4$, $P = 0.0145$). *, comparison with nicotine-alone group.

1992). Using different pharmacological tools, we have determined whether the nAChRs containing the highly calcium-permeable α7 subunit contribute to the stimulation of mIPSCs by nicotine. MLA acts noncompetitively at α7 subunit containing nAChRs and 100 nM is sufficient to block these receptors in cultured hippocampal neurons (Alkondon and Albuquerque 1993). We first co-applied nicotine with 100 nM MLA and observed no significant difference in the nicotine-stimulated mIPSC frequency (robust stimulation was still induced; $P = 0.40$, $n = 6$; Fig. 5, *Ab* and *B*). The low-affinity α7 agonist 10 mM choline (Alkondon et al. 1999) failed to enhance mIPSC frequency (baseline frequency = 0.47 ± 0.05, peak frequency during choline application = 0.78 ± 0.10, $n = 4$, $P = 0.17$). In contrast, the relatively nonselective nAChR antagonist, 1 μM MEC was able to antagonize ~90% of the effect of nicotine ($P = 0.019$, $n = 4$; Fig. 5, *Aa* and *B*). The lack of a α7 agonist effect (and only partial antagonist effect), but the apparent requirement for calcium entry through nAChRs (as opposed to activation of VSCCs secondary to nicotinic depolarization) suggested the involvement of other calcium-permeable nAChRs subtypes (see DISCUSSION).

Effect of nicotine on spontaneous inhibitory synaptic transmission

Nicotine-stimulated mIPSCs could come from at least two populations of striatal GABAergic terminals, from fast-spiking (FS) intrinsic GABAergic interneurons and/or MSNs (Czubayko and Plenz 2002; Guzman et al. 2003; Koos and Tepper 1999, 2002; Koos et al. 2004; Tunstall et al. 2002). Striatal FS-interneurons make multiple contacts on MSNs and their eIPSCs are more than four times larger than the quantal amplitude estimated from mean-variance analysis. In the case of MSN–MSN pairs, the eIPSC and the quantal current are similar in amplitude (Koos et al. 2004). Therefore if MSNs were to spontaneously fire, the resulting sIPSC would have the same size as the mIPSC. Assuming MSN input-derived sIPSCs are largely uniquantal, we would not expect an increase in their firing rate to be associated with an increase in sIPSC amplitude. Thus we monitored nicotine-induced sIPSCs in the absence of TTX to determine whether striatal FS interneurons were excited by nicotine and formed synapses on MSNs. We determined whether the amplitude of MSN sIPSCs increased (compared with mIPSCs), reflecting AP-mediated synchronized release of GABA from multiple FS-neuron terminals. In the absence of TTX, we observed a significant increase in sIPSC frequency and amplitude during nicotine treatment ($n = 3$) (Fig. 6, *A* and *B*) consistent with activation of multiple release sites on intrinsic interneuron axons. To further explore the mechanism of the sIPSC changes, we compared the rising phase of sIPSCs before and after nicotine application (> 20 pA sIPSCs, $n = 36$ control and 53 nicotine; Fig. 6*C, a* and *b*). We did not observe differences in kinetics or notches on the rising phase of single sweeps (indicating asynchronous release of quanta) (Xiang and Brown 1998), suggesting that sIPSCs reflect synaptic vesicles that were synchronously released with nicotine stimulation presumably from the stochastic firing of individual FS interneurons and not MSNs (see DISCUSSION). We believe that FS interneurons are the likely mediator of the nicotine effect based on the previous observation that interneuron-driven sIPSCs are much larger than those driven by MSN–MSN synapses (Koos et al. 2004). The observed increase in both sIPSC amplitude and frequency could only be attributed to an increase in FS-interneuron firing (which make multiple contacts with MSNs) or possibly the quantal content at each MSN or FS neuron terminal.

To further determine the nature of sIPSCs in striatum, we tested the sensitivity of nicotine-induced sIPSCs increase to 1 μM MEC, a nonselective nAChR antagonist. Similar to its effect on mIPSCs, 10-min perfusion of 1 μM MEC prior to nicotine application (in the continued presence of MEC) completely suppressed the nicotine effect on sIPSC frequency (fold increase = 1.8 ± 0.3, $n = 8$, $P < 0.001$ as compared with

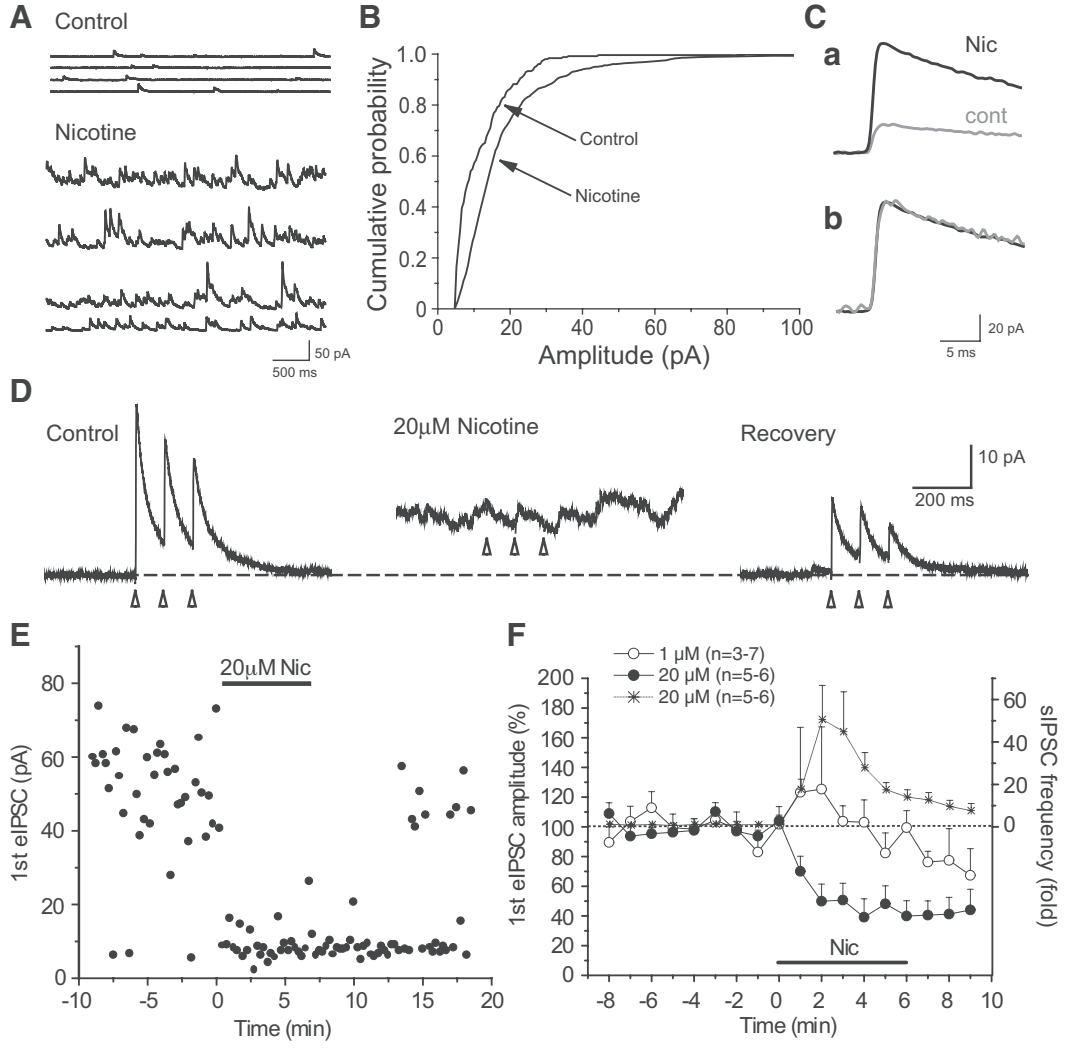

FIG. 6. Nicotinic modulation of eIPSCs. Neurons were held at 0 mV to produce an outward going IPSC mediated by GABA receptors (100 μM APV and 10 μM CNQX included). *A*: nicotine enhanced the frequency and amplitude of sIPSCs. Representative traces of sIPSCs were recorded in the presence of 100 μM APV and 10 μM CNQX at 0-mV holding potential and with addition of 20 μM nicotine in the bath. *B*: cumulative probability histogram showing the sIPSC amplitude distribution under control conditions (255 events) and with 20 μM nicotine (1,026 events) from the same cell as in *A*. Significant difference in sIPSC amplitude was observed after nicotine treatment (KS-test; $P < 0.001$). *Ca*: representative averaged records showing sIPSC kinetics with (Nic; black trace) and without (Cont; gray trace) nicotine treatment. The Nic trace is an average of 53 sweeps that were larger than 20 pA (amplitude). The Cont trace is an average of 36 sweeps that were randomly chosen. *Cb*: 2 traces in Ca are normalized to the peak current for control and nicotine cases. Note rising phase is superimposable for both cases, suggesting the large responses were not induced by accumulation of multiple quanta. *D*: using a bipolar stimulus electrode placed in the striatum, IPSCs were evoked by giving 3 presynaptic stimuli at 100-ms intervals (*left*) every 20 s. Addition of 20 μM nicotine resulted in a large increase in membrane noise attributed to the robust increase in sIPSC rate and an accumulation of a steady outward current presumably composed of many mIPSCs (*middle*). After nicotine washout, responses were recovered partially (*right*). Each trace is an average of 20 sweeps. *E*: time course of the 1st eIPSC in the same cell in *D*. During 20 μM nicotine treatment the 3 stimuli failed to produce IPSC or they were greatly attenuated in amplitude. *F*: group data of the 1st eIPSC amplitude and sIPSC frequency plotted against time. Responses were averaged every 60 s and normalized to an average of 5-min period before nicotine application. In the presence of 20 μM nicotine (●) eIPSC was attenuated more than that in 1 μM nicotine (○). sIPSC frequency (*) was enhanced by 20 μM nicotine.

nicotine-alone group). The depolarization, observed during nicotine application in the presence of TTX, was also abolished by 1 μM MEC application (-75.9 ± 0.9 mV for control before MEC perfusion, -77.2 ± 0.2 mV for during MEC perfusion alone and -77.1 ± 0.3 mV for during nicotine with MEC).

Because neurons were routinely held at depolarized potentials for monitoring GABAergic sIPSCs, it was possible that nicotine treatment might also engage the phenomenon termed depolarization-induced suppression of inhibition (DSI) that was first observed at GABAergic synapses onto cerebellar Purkinje cells and hippocampal CA1 pyramidal neurons (Llano et al. 1991; Pitler and Alger 1992; Vincent et al. 1992). To rule out this possibility, we tested the nicotine effect at −65-mV holding potential in neurons that were filled with an intracellular solution containing high chloride to better monitor sIPSCs at negative holding potentials. Under these conditions, robust enhancement of sIPSC frequency by nicotine was still observed at a level similar ($P = 0.53$, $n = 4$, see supplementary Fig. 4) to the routinely applied condition (low chloride in the pipette with 0-mV holding). These results suggest that the depolarized holding potential in our experiments is not engaging forms of plasticity that modify the response to nicotine.

Effect of nicotine on evoked inhibitory synaptic transmission

To assess the effects of nicotine-stimulated mIPSC and sIPSC activity on evoked synaptic transmission, we recorded eIPSCs from MSNs while stimulating locally within the striatum under conditions that block excitatory synaptic transmission. Using this recording configuration under control conditions, we were able to produce eIPSCs in response to presynaptic stimulation. To assess changes in release probability, we used three pulses of presynaptic stimulation separated by 100 ms (Fig. 6*D*). In the presence of 1 or 20 μM nicotine ($n = 7$ and 6 cells, respectively), we observed no consistent change in paired-pulse ratio [2nd pulse amplitude/1st, pre vs. post nicotine application; 1.22 ± 0.76 vs. 1.58 ± 0.60; $P = 0.26$ (paired *t*-test) for 1 μM nicotine; 0.73 ± 0.30 vs. 1.37 ± 0.56; $P = 0.12$ (paired *t*-test) for 20 μM nicotine see supplementary Fig. 3]. Application of nicotine as in previous experiments was found to greatly elevate baseline mIPSC and sIPSC frequency (Figs. 2 and 6*A*). In the presence of this enhanced GABAergic activity, we observed a large reduction in eIPSC amplitude (Fig. 6, *D–F*). Furthermore, there were apparent presynaptic excitation failures induced by nicotine as many trials failed to evoke synaptic response. In analyzing these data, we first averaged all eIPSC traces (including failures). To better assess potential effects of increased presynaptic failure after nicotine, we also averaged responses with presumed successful stimulation during control periods, during nicotine application, and during nicotine washout (Fig. 6*E*). We found that even with exclusion of failures (successful responses only), nicotine treatment resulted in a very large decrease in eIPSC amplitude, indicating a large inhibitory effect of nicotine on evoked synaptic activity. Although the depression of eIPSC amplitude partially reversed after 10 min of nicotine washout, there was a persistent increase in response failures, indicating potential long-lasting presynaptic effects of nicotine. To assess the temporal relationship between nicotine effects on mini release and evoked release, we examined the frequency of sIPSCs (presumed minis and quanta released by spontaneous action potentials in the absence of TTX) during the baseline period preceding evoked release and observed a strong temporal relationship between depression of evoked release and enhancement of the spontaneous events (Fig. 6*F*). Furthermore comparison of nicotine stimulation data from different cells under conditions that either isolate minis or enable evoked release indicate that both effects are manifested quickly within tens of seconds (see Fig. 4*A* for mini time course of mIPSC elevation by nicotine). In addition to having a temporal link between high mini rates and depression of evoked release (when different cells are compared), we also find that 1 μM nicotine, which produces only a small increase in mIPSC frequency, also fails to depress eIPSCs, again suggesting parallels between the two processes (Fig. 6*F*). Although we observed a temporal correlation between the increase in sIPSC frequency and the depression of the eIPSCs, this correlation does not mean that increases in mini frequency are sufficient to block eIPSCs. Perhaps increases in mIPSC frequency and depression of evoked activity are triggered by similar processes such as presynaptic depolarization. We speculated that eIPSC depression after nicotine application may be due to the presynaptic depolarization that leads to sodium channel inactivation. To determine the role of mild nicotinic depolarization in the mechanism of eIPSC depression, we performed experiments with low concentrations of KCl in the ACSF (2.5, 5, 10, and 15 mM) to potentially mimic nicotine action. Only in the case of 15 mM KCl did we observe effects that resembled those of nicotine. KCl (15 mM) was iso-osmotically substituted for NaCl in the bathing solution, and the effect on eIPSC was assayed in the absence of nicotine and TTX. Five minutes of 15 mM KCl application was sufficient to cause complete loss of the eIPSC (156 ± 37 pA for control condition and 16 ± 12 pA for during 15 mM KCl, $n = 6$, $P < 0.001$, Fig. 7, *B* and *D*). Although the eIPSC was blocked, a robust nicotine effect on sIPSC frequency was observed (fold increase $= 15.4 \pm 3.4$, $n = 5$). This result suggested that nicotinic depolarization may be sufficient to both block eIPSCs and to increase mIPSC frequency. However, we found that the magnitude of somatic depolarization elicited by 15 mM KCl (27.1 ± 2.1 mV, $n = 6$, measured in current clamp, Fig. 7*A*) was well above the effect of nicotine on membrane potential. To reproduce a somatic depolarization comparable to that induced by nicotine, lower concentrations of KCl were tested. As compared with the normal KCl concentration (2.5 mM), 5 mM KCl led to 7.33 ± 0.67 mV ($n = 3$) of somatic depolarization (Fig. 7*A*) but did not significantly reduce the eIPSC (177.8 ± 64.1 to 144.8 ± 61.5 pA, $n = 8$, $P > 0.05$) or alter mini frequency (0.66 ± 0.19 to 0.92 ± 0.24 Hz, $n = 8$, $P > 0.05$). With 10 mM KCl, 19.2 ± 3.2 mV of somatic depolarization ($n = 3$) was observed with only a marginal effect on sIPSC frequency (fold increase $= 2.56 \pm 0.35$, $n = 6$, $P = 0.027$, Fig. 7*C*) and no significant effect on eIPSC amplitude (168 ± 54 to 106 ± 40 pA, $n = 5$, $P = 0.38$).

Dopaminergic modulation of striatal cholinergic enhancement of GABA activity

DA is known to modulate GABAergic transmission presynaptically in the striatum (Centonze et al. 2003; Cooper and Stanford 2001; Delgado et al. 2000; Guzman et al. 2003). To determine whether nicotine-induced GABA release might involve dopamine, we examined the effect of D1 and D2 dopamine receptor antagonists. A combination of D1 and D2 receptor antagonists, SCH23390 (10 μM) and sulpiride (50 μM), were perfused for 10 min prior to adding 20 μM nicotine in the continued presence of the antagonists. A reduction in nicotine-stimulated mIPSC frequency was observed when compared with the nicotine-alone group (fold increase $= 3.8 \pm 0.7$ for the dual dopamine blocker cocktail, $n = 5$, $P < 0.05$). To further determine the subtype of dopamine receptor, SCH23390 (10 μM) and sulpiride (50 μM) were tested separately. After 10-min perfusion of either SCH23390 or sulpiride, 20 μM nicotine was applied to the recording chamber. A reduction in nicotine-induced mIPSC frequency was detected when SCH23390 was administered alone (fold increase $= 4.7 \pm 0.6$, $n = 6$, $P < 0.05$ for comparison with nicotine-alone group). In contrast, perfusion of D2 receptor antagonist sulpiride (50 μM) did not alter the mIPSC frequency increase induced by nicotine (fold increase $= 13.4 \pm 2.1$, $n = 4$; $P = 0.66$ when compared with nicotine-alone group). Therefore the observed enhancement of GABAergic activity may in part be a result of elevated dopaminergic activity triggered by increased nicotinic activity. To more directly test this possibility, the D1 receptor agonist SKF38393 (10 μM) was perfused into

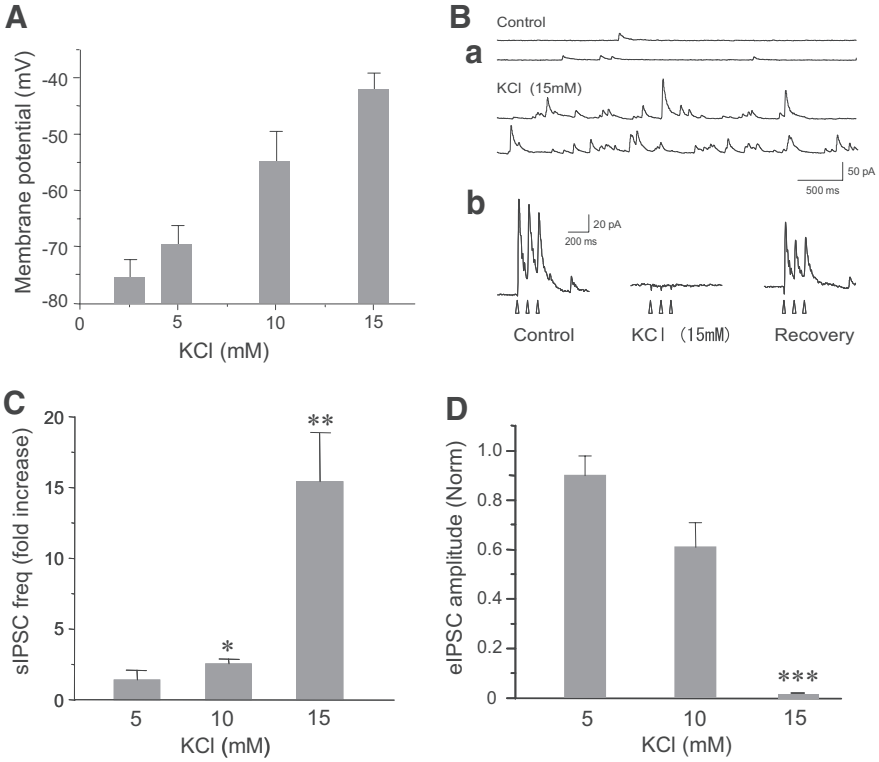

FIG. 7. Effect of low concentrations of KCl on sIPSCs and eIPSCs. Experiments were performed on striatal MSNs perfused with artificial cerebrospinal fluid (ACSF) containing 100 μM APV and 10 μM CNQX. *A*: membrane potentials were monitored at current-clamp mode in MSNs with 2.5, 5, 10, or 15 mM KCl. A stable membrane potential was 1st obtained for 10 min before altering KCl. The low KCl-containing ACSF was washed in 5–10 min until the membrane potential stabilized. *B*: example traces showing the effect of 15 mM KCl on sIPSC (*Ba*) and eIPSC (*Bb*). The stimulation scheme in *Bb* is the same as in Fig. 6. *C*: group data demonstrating that despite the large depolarization caused by 10 mM KCl, a robust enhancement in sIPSC frequency does not occur until 15 mM KCl. *D*: group data showing the eIPSCs diminishing with increasing concentration of KCl. Note: these observations were made in the absence of TTX and comparisons were made between elevated KCl group and normal KCl group.

the recording chamber for 10 min. To our surprise, no change in mini frequency was observed ($P = 0.77$ when comparison was made between baseline mIPSC frequency and frequency during SKF38393 application, Fig. 8, *Ae* and *Ba*). These results indicate that D1 receptor activity is not directly linked to mIPSC stimulation but may somehow be permissive for the process. A observation of the D1 receptor's influence on nicotine-induced GABA release has been reported in substantia nigra (Kayadjanian et al. 1994).

To rule out direct competition between 20 μM nicotine and DAergic antagonists, we tried the same experiment with 100 μM nicotine in the presence of 10 μM SCH23390 and 50 μM sulpiride. Compared with 20 μM nicotine, 100 μM nicotine induced a significantly larger increase in mIPSC frequency (fold increase = 27.1 $\pm$ 5.8, $n = 5$, $P < 0.01$). Similar to data acquired with 20 μM nicotine application, perfusion of D1 antagonist SCH23390 caused a decrease in 100 μM nicotine-induced facilitation of mIPSC frequency (fold increase = 10.9 $\pm$ 3, $n = 5$, $P < 0.05$ for comparison with 100 μM nicotine-alone group). Nevertheless, even in the presence of both D1 and D2 antagonists, SCH23390 (10 μM) and sulpiride (50 μM), 100 μM nicotine application led to a reduced enhancement of GABAergic activity as compared with 100 μM nicotine-alone group (fold increase = 10.4 $\pm$ 3.58, $n = 5$, $P < 0.05$), confirming the data obtained with 20 μM nicotine. Overall our data suggest that D1 but not D2 receptors may impose an indirect influence on cholinergic modulation of striatal GABA activity.

DISCUSSION

We report that nicotine can lead to a robust increase in mIPSC frequency in striatal MSNs. The robust increase in mIPSC frequency was induced by a calcium influx through calcium-permeable nAChRs and not VSCCs. Interestingly, striatal MSN mEPSCs were not enhanced in frequency or amplitude, adding selectivity to nicotinic modulation of striatal miniature activity in contrast to lateral geniculate nucleus where nicotine enhances both types of miniature activity (Guo et al. 1998).

Presynaptic nAChRs enhance GABAergic transmission in a calcium-dependent manner

Previous work has shown evidence for the existence of presynaptic nAChRs on GABAergic neurons. Léna and Changeux (1997) concluded that nicotine-stimulated GABA release from mouse thalamus occurs via activation of nAChRs on the nerve terminal based on the observation that nicotinic effects were TTX-insensitive. Nicotinic agonists also induce [^{3}H]-GABA release from isolated striatal synaptosomes (Behrends and ten Bruggencate 1998). Nicotine-stimulated changes in $[Ca^{2+}]_i$ were observed in synaptosomes prepared from striatum and were insensitive to VSCC blocking toxins, suggesting calcium entry through calcium-permeable nAChRs (Nayak et al. 2001). These reports are consistent with our observation that mIPSC frequency in striatum was enhanced by nicotine in a TTX-insensitive and VSCC-independent manner. Preliminary experiments using blockers of intracellular calcium stores including ryanodine, cyclothiazide, or thapsigargin had no effect on nicotine stimulated mIPSC frequency. Positive control experiments with caffeine are not feasible because caffeine has been shown to block GABAergic activity in hippocampal slices (Taketo et al. 2004), consistent with our results (data not shown). In contrast to these studies, Sharma and Vijayaraghavan (2003) demonstrated that nicotine-enhanced mEPSC frequency and amplitude in CA3 pyramidal neurons resulted from calcium release from a ryanodine-sensitive store after calcium

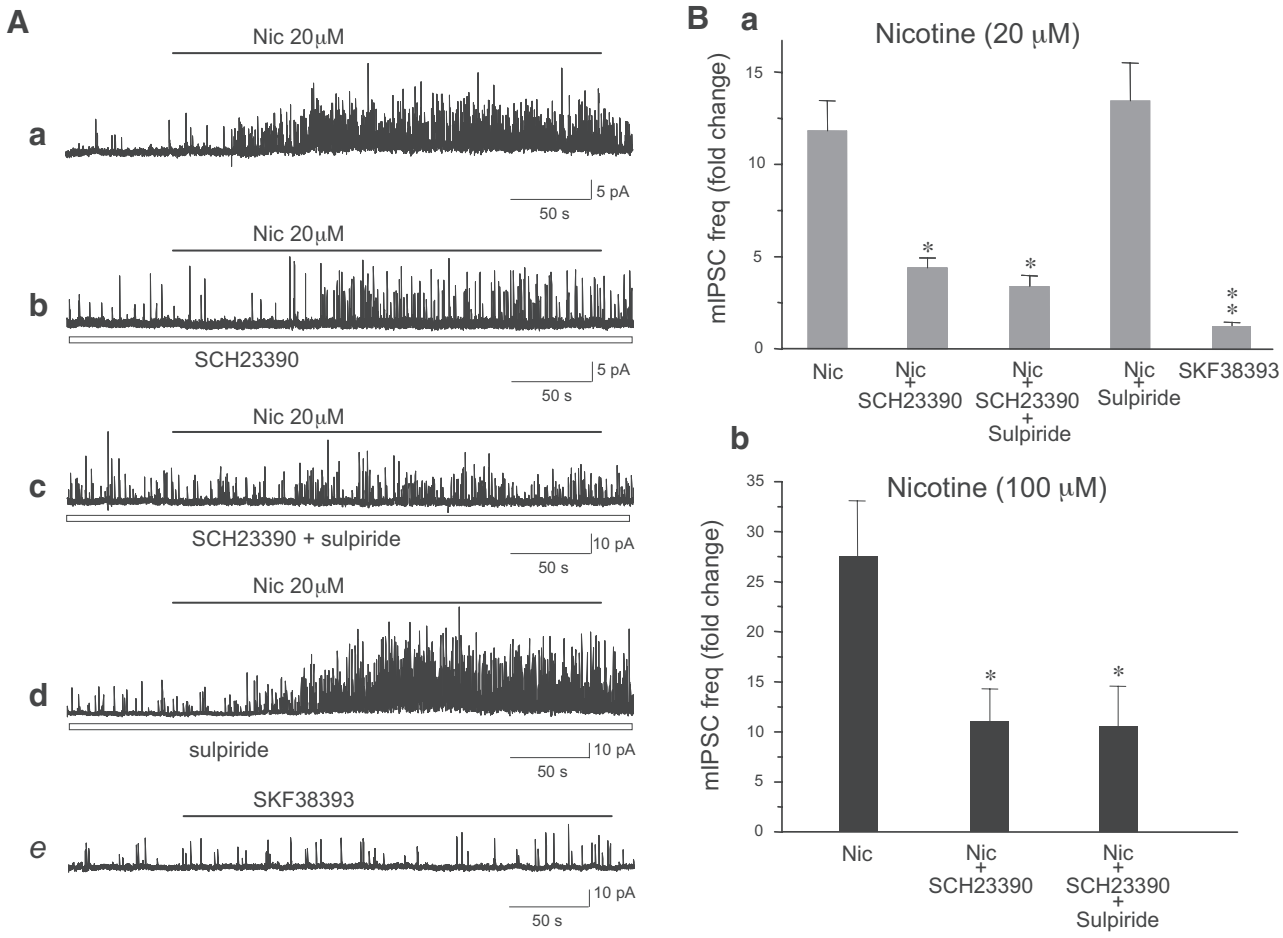

FIG. 8. Effect of dopaminergic activity on mIPSC stimulation by nicotine. *A*: representative traces during pharmacological manipulation of nicotine-induced mIPSCs. The perfusate contains: 20 μM nicotine alone (*Aa*), 20 μM nicotine +10 μM SCH23390 (*Ab*), 20 μM nicotine +10 μM SCH23390 + 50 μM sulpiride (*Ac*), 20 μM nicotine +50 μM sulpiride (*Ad*), and lastly 10 μM SKF38393 alone (*Ae*). Note: none of the dopaminergic drugs changed the baseline frequency of mIPSCs. *B*: group data showing the effect of SCH23390, sulpiride, and SKF38393 on nicotine-induced mIPSC enhancement. The reduction of nicotine (20 μM) effect by SCH23390 as shown in Ba was also observed with 100 μM nicotine for Nic+SCH23390 group and Nic+SCH23390+sulpiride group as shown in *Bb*. Note: in *Ba,* SKF38393 application was performed in the absence of nicotine. *, comparison with the nicotine-alone group (the 1st bar).

influx through α7-type αBgt-sensitive nAChRs on mossy fiber terminals. Large terminals such as mossy fibers contain αBgt-sensitive (α7) nAChRs that desensitize rapidly (Couturier et al. 1990; Zhang et al. 1994). This recruitment of calcium stores would then amplify and prolong the enhanced miniature synaptic activity. In contrast, in relatively smaller terminals like GABAergic interneurons in striatum (Kubota and Kawaguchi 2000), accumulation of calcium in the terminals caused by a continuous calcium influx via non-α7 nAChRs could be sufficient to induce the pronounced elevation in mIPSC frequency.

Possible subtypes of presynaptic nAChRs

Several nAChRs subunits (α2–7, β2–4) are found in the mouse and rat striatum (Champtiaux et al. 2003; Nayak et al. 2001; Zoli et al. 2002). Although this suggests that these subunits are on presynaptic terminals within the striatum, it is not clear which cell types contain these subunits because a heterogeneous group of presynaptic terminals is also present including inputs from extrinsic DAergic and glutamatergic neurons and intrinsic cholinergic and GABAergic neurons. Champtiaux et al. (2003) and Zoli et al. (2002) showed α6 and β3 subunits of nAChRs were located on DA terminals and α4 and β2 subunits were both expressed on DA terminals and non-DA cells or terminals. No evidence was found for α7 subunits on GABAergic neurons.

Functional nAChRs have been shown on striatal glutamate terminals in vivo (Garcia-Munoz et al. 1996) and in vitro (Kaiser and Wonnacott 2000; Wonnacott et al. 2000). Kaiser and Wonnacott (2000) suggest that these striatal nAChRs contain α7 subunits. If sufficient numbers of α7 nAChRs are on glutamatergic terminals, we should have detected an enhancement of mEPSC frequency by nicotinic agonist. However, because adult rats were used in the experiments mentioned in the preceding text, it is conceivable that we did not detect nAChRs responses of α7 pharmacology because we used slices prepared from young rats. However, the rapid desensitization of α7-containing nAChRs makes it difficult to exclude their involvement in mEPSC enhancement by nicotine application.

Our observations with nicotinic reagents suggest that GABAergic interneurons express nAChRs coupled to mIPSC stimulation that are likely composed of non-α7 subunits because neither an α7-selective antagonist MLA, nor agonist,

choline, exhibited any effect (Fig. 5). However, we have to consider the experimental limitation of our relatively slow perfusion system. Without a sufficiently rapid perfusion system, the fast nicotinic component could be reduced (Zhang et al. 1994). Our observation that nicotine's effect on mIPSCs can be rescued from 0 calcium suppression by switching to nicotine-containing normal calcium ACSF suggests that the rapidly desensitizing component of the nicotinic current may not be required for modulation of GABAergic mIPSCs. If nicotine's effect relied on the initial fast component (possibly mediated by α7-containing receptors), we would not expect to see a rescued nicotine response in normal calcium ACSF after prolonged exposure to agonist. Taken together these data help support our proposal that non-α7 subunits are likely involved in the enhancement of mIPSCs we observe. Further pharmacological analysis and/or use of knockout animals will be necessary to identify the exact subtype of nicotinic receptor involved.

nAChRs on GABAergic interneuron terminals trigger enhanced mIPSCs

FS interneurons in striatum are able to induce ACh-sensitive APs (Koos and Tepper 2002) and make multiple synaptic contacts on MSNs (Koos et al. 2004). In our study, we observed that nicotine application produced increases in sIPSC amplitude but not mIPSC amplitude;, it is thus likely that at least a part of this effect is mediated by activation of FS interneurons. However, there are other types of GABAergic interneurons; persistent and low-threshold spike (PLTS) neurons and calretinin-colocalized neurons are present in the striatum (Kawaguchi 1993; Kubota and Kawaguchi 2000; Rymar et al. 2004) and could also contribute to the increase in sIPSC amplitude. Reports based on anatomical (Tepper et al. 1998) and electrophysiological studies (Koos et al. 2004) indicate that synaptic connections between MSNs (MSN to MSN) are rare in slices prepared from animals younger than postnatal day 15. Because we used animals of this age range and routinely observed increases in GABAergic transmission by nicotinic agonists, we do not think that MSNs are the major source of mIPSCs. MSNs are also unlikely to be a major source of nicotine-stimulated sIPSCs because we only detected a modest subthreshold depolarization of MSNs with nicotine application (Fig. 1*Ba*). Therefore nicotine-stimulated mIPSCs recorded on MSNs could mostly come from the terminals of striatal GABAergic interneurons, like FS interneurons.

Possible mechanism of nicotine effect on evoked GABAergic synaptic transmission

Nicotine was found to produce a robust change in mIPSC frequency, which at times was accompanied by diminished eIPSCs (Fig. 6). There are two possible mechanisms that might be responsible for diminished eIPSCs after nicotine enhancement of GABA mIPSCs. First, this effect might result from an occlusion of eIPSCs by depletion of transmitter after robust nicotine-stimulated mIPSCs and sIPSCs. However, assuming nicotine affects all synapses equally, it may be difficult for readily releasable pool to be depleted by nicotine stimulation of release. The nicotine-stimulated release rate at each synapse would be well $<$0.01Hz, given a conservative assumption of 500 synapses on each neuron (Guzman et al. 2003; Koos et al. 2004). Because MSNs can normally follow these frequencies, we would not expect transmission to be blocked.

Another possibility is that the increased excitability and sustained depolarization of inhibitory neurons by nicotine induce inactivation of Na^+ channels leading to a blockade of eIPSCs. Sustained depolarization by 15 mM KCl produced a comparable result as nicotine, i.e., enhancement of mini sIPSC frequency and profound reduction of eIPSC amplitude. In contrast, 5 mM KCl neither affected mini frequency nor altered amplitude of eIPSC with comparable levels of depolarization as seen with nicotine application. The observation that higher levels of somatic depolarization were produced by 15 mM KCl (than nicotine) would suggest that depolarization induced sodium channel inactivation is not responsible for the nicotine-induced increase in GABA release. However, it needs to be noted that our current-clamp recordings of nicotine-induced depolarization were performed on MSN somata instead of interneuron terminals. It is conceivable that interneuron terminals or axons may undergo stronger depolarization with nicotine leading to conduction block.

Indirect influence of dopamine D1 receptors on nicotine stimulation of GABA mIPSCs

Dopamine plays a critical role in striatal function as abnormal nigrostriatal projection has been implicated in diseases such as Parkinson disease both in human and in animal models (Bergman et al. 1998; Berke and Hyman 2000). In addition to targeting to major striatal projection neurons, dopaminergic inputs also terminate on striatal interneurons, where identification of receptor subtype has been complicated (Bracci et al. 2002; Centonze et al. 2003). It is possible that the observed enhancement of GABA mIPSC frequency by nicotine is mediated in part by dopaminergic neurons. To rule out an indirect effect of nicotine on mIPSC frequency through stimulation of DA release, we have performed a series of pharmacological experiments with D1 and D2 antagonists and a D1 agonist. Our results show that the D1 antagonist SCH23390 partially blocked the enhancement of GABA mIPSCs by nicotine. If dopaminergic terminals were excited by nicotine leading to dopamine release, we would expect that a D1 agonist should mimic nicotine's effect and increase mIPSC frequency. Failure of the D1 agonist SKF38393 to induce enhancement of GABA mIPSCs suggests that the action of D1 antagonists is likely an indirect effect. Our negative data with D1 agonist suggest that a direct excitatory effect of dopamine on striatal GABAergic interneurons are not sufficient to facilitate mIPSC frequency in MSNs (Aosaki et al. 1998; Bracci et al. 2002). However, we do not exclude the possibility that D1 receptors may in some way affect GABAergic mIPSCs stimulated by nicotine.

Physiological function of nicotine-stimulated mIPSCs

One remaining question with our study concerns the relationship between the Cl^- equilibrium potential and possible depolarizing or hyperpolarizing effects of nicotine-stimulated mIPSCs. In vivo (Mercuri et al. 1991) and in acute slices the reversal potential for GABA responses was measured at approximately -60 mV with intracellular recording (Jiang and North 1991; Kita 1996; Koos and Tepper 1999) and at -64

mV with gramicidin-perforated patch (Bracci and Panzeri 2006). In our experiments, we used Cl^- concentrations that led to a −58-mV reversal potential. It is conceivable that this reversal potential may be developmentally regulated or even different for dendritic versus somatic compartments. Therefore it is unclear whether nicotine-stimulated mIPSCs would depolarize the actual membrane potential of MSNs. The ability of nicotine to robustly increase mIPSC rates to a point where they begin to affect evoked synaptic activity suggest that nicotine could be involved in changing the mode of inhibition in MSNs from a phasic to a tonic one.

These findings raise the more general question of functional significance of minis. Perhaps scenarios exist in vivo where endogenous presynaptic modulators can increase minis to high levels. Using in vivo dialysis of TTX and intracellular recordings, Pare et al. (1997) observed that relatively intense periods of minis (~10 Hz) are common and contribute to regulation of baseline synaptic parameters such as input resistance. Assuming that a single mini is insufficient to produce AP firing by itself, conditions that promote the spatial and temporal summation of multiple synaptic inputs could more effectively modulate firing. For example, tonic miniature GABA release occurs preferentially at sites close to the AP initiation site in dentate gyrus granule cells and is likely to regulate their firing (Claiborne et al. 1986). In the CA3 region of hippocampus, mossy fibers form synapses onto the proximal dendrites of CA3 pyramidal neurons (Ishizuka et al. 1990). Given that these synaptic locations are relatively close to the AP trigger zones, nicotine-stimulated minis might easily lead to firing even in the presence of GABAergic inhibition (Sharma and Vijayaraghavan 2003). Interestingly, current injection experiments indicate that firing of electrically compact interneurons can be influenced by individual minis (Carter and Regehr 2002).

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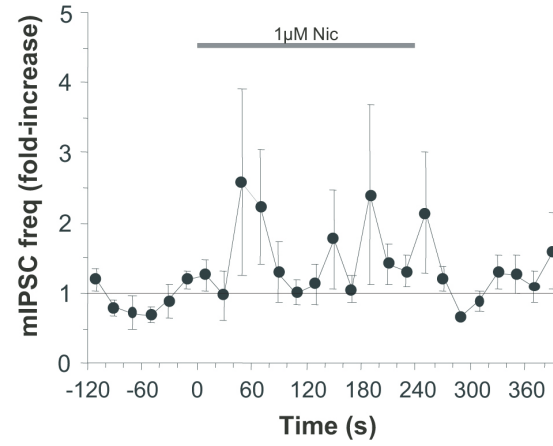
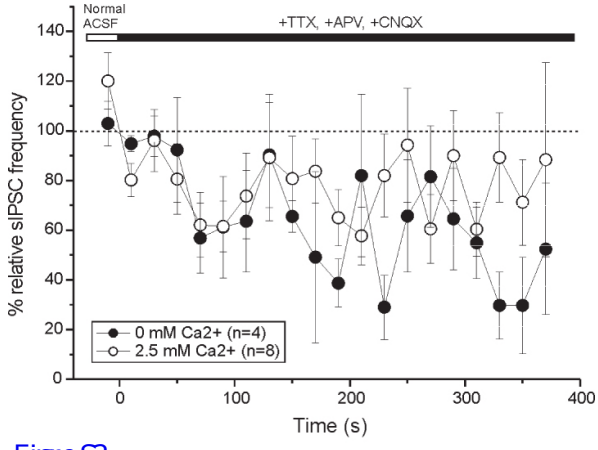
Supplemental Figures



- Figure S1



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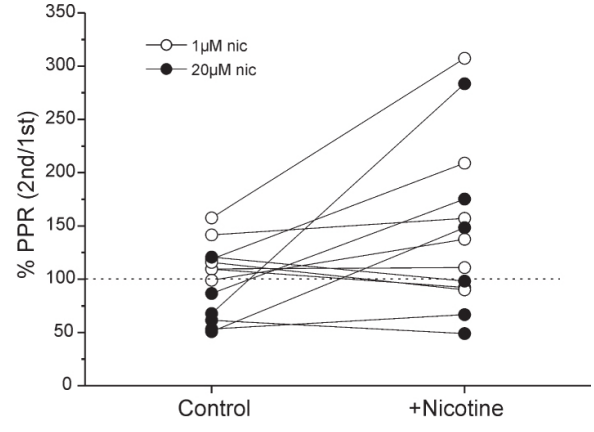

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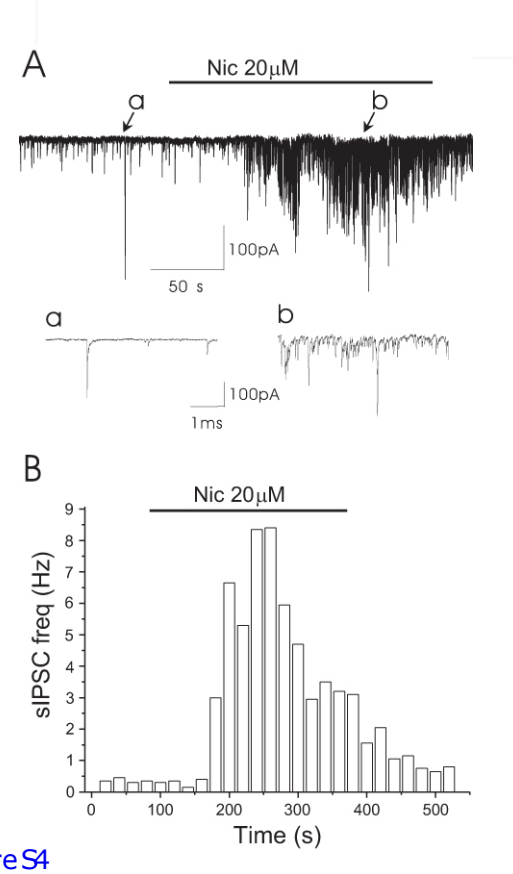

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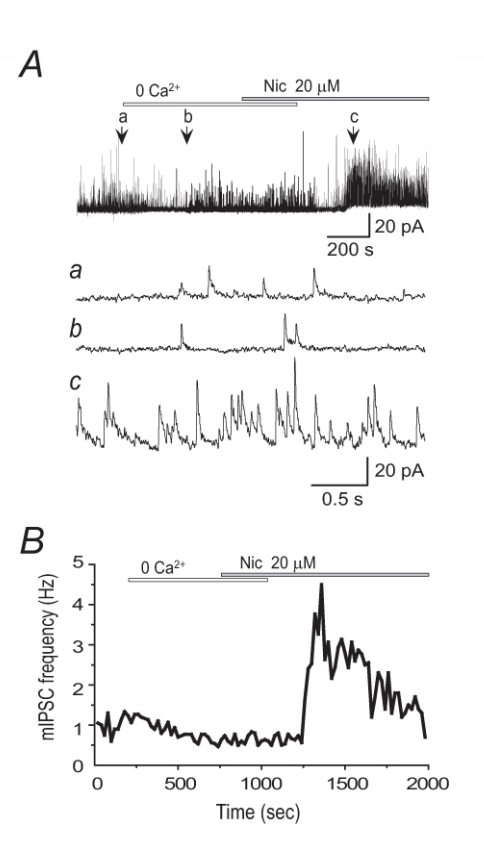

- Figure S5