Differential Modulation by Nicotine of Substantia Nigra Versus Ventral Tegmental Area Dopamine Neurons

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INTRODUCTION

Dopaminergic transmission from substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) are involved in motor control and motivation, respectively (Di Chiara and Imperato 1988; Hornykiewicz 1966; Koob and Nestler 1997; Schilstrom et al. 1998). Dopamine neurons in these two nuclei share many physiological properties (Grace and Onn 1989; Johnson and North 1992; Lacey et al. 1989), but they differ markedly in their sensitivity to addictive drugs, such as nicotine. Ultimately, drug-induced dopamine release from these nuclei is due to the intrinsic sensitivity of the dopamine neurons as well as their synaptic inputs.

Both these nuclei contain dopaminergic and GABAergic cell types (Johnson and North 1992; Lacey et al. 1989) and express similar cohorts of nicotinic acetylcholine receptor (nAChR) subunits (Charpantier et al. 1998; Klink et al. 2001; Pidoplichko et al. 1997; Wada et al. 1989). Recent investigations have demonstrated that the dopamine neurons in VTA express higher levels of nAChRs than those in SNc (Klink et al. 2001; Wooltorton et al. 2003), and although these findings constitute a key element in the dopamine output, these studies did not examine potential differences in nAChR expression on the synaptic inputs to the dopamine neurons.

We previously found that physiologically relevant nicotine levels enhance excitatory glutamatergic inputs to VTA DA neurons by activating presynaptic α7 nAChRs (Mansvelder and McGehee 2000). Nicotine also modulates inhibitory inputs by first activating and then desensitizing non-α7 nAChRs found on the GABAergic cells, which reduces the inhibitory drive to the postsynaptic DA neurons. Thus the combination of enhanced excitation and decreased inhibition contributes to a net excitation of VTA DA neurons in the presence of nicotine (Mansvelder et al. 2002). In this study, we sought to explore the differential nicotinic sensitivity of the excitatory and inhibitory synaptic inputs to dopamine neurons between the SNc and VTA.

METHODS

Tissue preparation

Horizontal brain slices were prepared from Sprague Dawley rats (9–14 days old). After isoflurane anesthesia and rapid decapitation, the brain was removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 2.5 KCl, 1 MgCl2, 6 H2O; 2.5 CaCl2, 20 glucose, 1 NaH2PO4, 25 NaHCO3, and 1 ascorbic acid; bubbled continuously with 95% O2-5% CO2 for 10 min. The midbrain was cut away at the level of the fourth ventricle, and the brain was sliced using a vibromate. 250- to 300-μm slices containing SNc were incubated in oxygenated ACSF (32–34°C) for ≥1 h. For recording, the slices were transferred to a chamber superfused (~2 ml/min) with ACSF that did not include ascorbic acid. Experiments were carried out at room temperature.

Whole cell recording

Cells were visualized under infrared illumination using an upright microscope (Axioskop, Zeiss). Whole cell patch-clamp recordings of spontaneous excitatory postsynaptic currents (sEPSCs) were carried out in the presence of 20 μM bicuculline to block GABAA receptors. Electodes (2–7 MΩ) contained (in mM) 154 K-gluconate, 1 KCl, 1 EGTA, 10 HEPES, 10 glucose, and 5 ATP and 100 μM GTP. When recording spontaneous inhibitory postsynaptic currents (sIPSCs), 10 μM 6,6-dinitroquinoxaline-2, 3(1H, 4H) dion (DNQX) was included in the bath solution to block non-NMDA glutamate receptors and the internal solution was adjusted to 78 mM K-glucocolate and 77 mM KCl.


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to reverse Cl− flux in response to GABA_A receptor stimulation. Series resistance was 5–20 MΩ.

Hyperpolarization-induced inward currents (I_{H}) were assessed using 2-s voltage steps to negative potentials from a holding potential of −50 mV. Cells expressing I_{H}>25 pA at −120 mV were considered I_{H}−positive. Expression of this current has been associated with a dopaminergic phenotype in the SNC and VTA and is commonly used to identify DA neurons (Johnson and North 1992; Mercuri et al. 1995; Pidoplichko et al. 1997). Although this criterion has held up to scrutiny in the SNC, recent findings suggest that non-DA neurons in the VTA may also express this current (Ford et al. 2006; Margolis et al. 2006). Cell morphology has some predictive value for identifying dopaminergic cells, but it is not entirely reliable (Margolis et al. 2006). Based on the combined morphology and I_{H} of the neurons examined in our previous study, however, we contend that the VTA neurons discussed in this study are predominantly DA neurons.

Neurons were held at V_m = −50 mV to determine the presence of I_{H} current but were held at V_m = −70 mV for the other experiments. Recordings were filtered at 1 kHz and digitized at 5 kHz.

Drug application

All compounds were applied through bath perfusion with the exception of ACh and choline, which were applied by a focal puff with the exception of nicotine and ACh, which were applied by a focal puff pipette application system (Picospritzer, General Valve). The puff experiments utilized theta tubing pulled to a tip diameter of ~6 μm. Each side of the tubing was back-filled with ACSF containing either 1 mM ACh or 10 mM choline. The system allowed independent control of the ACh and choline channels and provided equal pressures to both. Focal application of saturating concentrations of ACh (1 mM) and choline (10 mM) were applied for 300-ms duration. Bath and drug solutions contained 10 mM DNQX and 20 μM bicuculline to block the major excitatory and inhibitory inputs to the cells. We saw no evidence of fast synaptic transmission under these conditions. Agonists were applied twice to each cell in an alternating fashion at 1-min intervals. There was no desensitization or cross-desensitization when the agonist applications were timed in this manner. Multiple sessions of agonist application were performed at successively increasing pressures to optimize the response of the cells. All antagonists and channel blockers were in the bath ≥15 min prior to application of nicotine, ACh, or choline. In the experiments that utilized TTX, voltage-gated Na^+ currents were monitored and the synaptic studies were not initiated until these currents blocked by >90%.

α7 and non-α7 receptors were blocked with 10 mM methylacelone (MLA) and 1 μM mecamylamine (MEC), respectively. Our studies in the MLA (Mansvelder and Gechee 2000; Mansvelder et al. 2002) suggest that these antagonists are sufficiently selective at these concentrations to separate the currents through their targeted receptors.

Data analysis

Peak whole cell responses to ACh or choline were averaged from at least two focal applications, and these averages were normalized to cell capacitance. Responses were not included in the analysis unless they exceeded five times the root mean square (RMS) of the baseline noise, as determined by the MiniAnalysis software (Synaptosoft). The ratio of ACh/choline response magnitude was then calculated for each cell as an estimate of the relative contribution of α7 nAChRs to the receptor population.

Spontaneous postsynaptic currents were analyzed off-line using the Mini Analysis Program and selected using amplitude, rise time, and area criteria. Amplitude thresholds were determined to be at least five times RMS of baseline noise. Each event was visually inspected to eliminate false positives caused by intermittent noise. After selection, Poisson regression analysis was employed to compare control and nicotine periods for each recording using STATA6 (Stata, College Station, TX). “Responsive cells” were defined as those that showed significant differences between control and experimental time periods (P < 0.05). A χ² test was used for comparison of response prevalence between SNC and VTA (2-tailed) as well as comparison response prevalence in the presence and absence of nAChR antagonists (1-tailed). Cumulative amplitude distributions were compared using the Kolmogorov–Smirnov test in SYSTAT (SPSS). Statistical differences in the variance of ACh and choline responses were determined using an F-test. Unless otherwise noted, data are presented as means ± SE.

Immunohistochemistry

To confirm that I_{H}-positive cells were indeed dopaminergic, three recorded neurons were filled with biocytin (2 mg/ml) via the recording electrodes and successfully identified using a Cy-3-conjugated streptavidin after immunostaining for tyrosine hydroxylase (TH) using TH-specific antibodies (Chemicon, Temecula, CA). After recording, tissue sections were fixed for 12–14 h in a 0.2 M phosphate buffer with 4% paraformaldehyde and 15% saturated picric acid at 4°C. The tissue was stored in 0.05 M Tris-buffered saline solution (TBS; pH = 7.4) at 4°C until processing. After permeabilization and blocking with normal goat serum (NGS; 4 × 10 min), tissue sections were treated with primary antibody rabbit anti-TH (Chemicon, 1:1,000) for 5 days at 4°C. After washing, the tissue was treated with an Alexa Fluor 488-labeled secondary antibody (Molecular Probes, 1:100), and Cy3-conjugated Streptavidin (Jackson ImmunoResearch, 1:500) for 2 h at room temperature. After additional rinses tissue sections were slide mounted and visualized using an Olympus Fluoview 200 laser scanning confocal microscope using Fluoview 2.1 graphics software. Omission of the primary antibody resulted in the absence of TH staining.

Chemicals

All chemicals were obtained from Sigma/RBI (St. Louis, MO) with the exception of TTX, which was from Alamone Labs (Israel), and DNQX, and 2-amino-5-phosphonovalerate (APV), which were from Tocris (Ellisville, MO).

RESULTS

Glutamatergic and GABAergic afferents provide the main excitatory and inhibitory input to the SNC and VTA (Bezd and Gross 1998; Charara et al. 1996; Grofova et al. 1982; Kalivas et al. 1993; Naito and Kita 1994; Ribak et al. 1980; Smith and Bolam 1990; Smith et al. 1996; Walaas and Fonnun 1980). Nicotine’s effect on these inputs, and on the DA neurons themselves, was examined in horizontal slices from neonatal rats (P9-14) that included the SNC and its afferent inputs. Whole cell patch recordings from dopaminergic neurons (Fig. 1A) were performed to test the effects of nicotine on EPSCs, IPSCs, and nicotinic receptor currents. The expression of a hyperpolarization-induced current (I_{H}) along with cell size and morphological criteria were used as indicators of dopaminergic phenotype (Fig. 1B). Post recording histology indicated that I_{H}-positive cells express the DA synthetic enzyme, tyrosine hydroxylase, which is consistent with previous studies showing that I_{H}-positive cells are dopaminergic (Fig. 1, A and B; n = 3) (Johnson and North 1992; Mercuri et al. 1995; Pidoplichko et al. 1997). I_{H}-negative cells were considered to be GABAergic cells (Fig. 1C).

Modulation of excitatory inputs by nicotine

In other brain regions, nicotine has been shown to increase excitatory synaptic drive by activating nAChRs on presynaptic...
glutamate terminals (Alkondon et al. 1996; Barazangi and Rode 2001; Gray et al. 1996; Mansvelder and McGehee 2000; McGehee et al. 1995; Vidal and Changeux 1993). The SNc receives glutamatergic afferents from local GABAergic neurons, the nucleus accumbens, the striatum, and the globus pallidus (Grofova et al. 1982; Kalivas et al. 1993; Ribak et al. 1980; Sesack and Pickel 1992; Smith et al. 1996). We tested the effect of nicotine on the glutamatergic inputs to SNc in horizontal slices. The GABA\textsubscript{A} antagonist bicuculline methiodide (20 \mu M) was included in the external solution to isolate excitatory inputs to the neurons. All neurons examined under these conditions displayed sEPSCs. Application of 1 \mu M nicotine to the slice resulted in an enhancement of glutamatergic transmission in a subset of cells tested, as shown by changes in sEPSC frequency (5 of 26, or 19%; Fig. 2, A and B). When nicotine did increase sEPSC frequency, no differences in the sEPSC amplitudes were seen between control and nicotine treatment (Fig. 2C). The increase in sEPSC frequency without a corresponding change in amplitude is consistent with a presynaptic modulation of glutamate release. Pretreatment with 1 \mu M TTX had no effect on the prevalence of nicotinic modulation of EPSC frequency (Fig. 2G), which further supports modulation via presynaptic nAChRs. There was, however, one cell in which the increase in sEPSC frequency was accompanied by a clear increase in amplitude (data not shown). We then normalized the frequency to the prenicotine baseline and averaged these values for all neurons that either showed a significant change in sEPSC frequency (5 of 26, or 19%) or did not respond to nicotine (Fig. 2E). For the responsive neurons, the magnitude of the change in sEPSC frequency was comparable to that observed previously in the VTA (Fig. 2F) (Mansvelder and McGehee 2000).

Although the enhancement of sEPSC frequency in the SNc is similar in magnitude to that observed in the VTA, the pharmacology of the nAChRs underlying this increase, along with the prevalence of the response were quite different (Fig. 2G). As noted earlier, 1 \mu M nicotine caused a significant increase in sEPSC frequency in 5/26 or 19% of DA cells tested in the SNc. This is significantly less than the 13/21 or 62% responsive cells seen in the VTA (P < 0.01).

Nicotinic enhancement of excitatory synaptic input to SNc DA neurons was not blocked by individual application of either 10 nM MLA, a selective \alpha\textsubscript{7} nAChR antagonist, or 1 \mu M MEC, a non-\alpha\textsubscript{7} nAChR antagonist. Rather a combination of the two antagonists inhibited nicotine-induced increases in sEPSC frequency (Fig. 3G), suggesting that both \alpha\textsubscript{7} and non-\alpha\textsubscript{7} receptors underlie this effect in SNc. This is not what is observed in the VTA, where MLA pretreatment inhibits nicotinic modulation of excitatory drive, suggesting a key role for \alpha\textsubscript{7} nAChRs at those synapses (Mansvelder and McGehee 2000).

**Modulation of inhibitory inputs by nicotine**

We also examined the effect of nicotine on the inhibitory inputs to SNc DA neurons. The SNc receives inhibitory afferents from local GABAergic neurons, the nucleus accumbens, the striatum, and the globus pallidus (Grofova et al. 1982; Kalivas et al. 1993; Ribak et al. 1980; Sesack and Pickel 1992; Smith and Bolam 1990; Walaas and Fonnun 1980). Brain slices were perfused with an ACSF solution containing 10 \mu M DNQX to block glutamatergic transmission. The Cl\textsuperscript{−} levels of the internal solution were also adjusted to improve resolution of GABAergic currents. Under these conditions, all synaptic activity was inhibited by bicuculline (20 \mu M). When bicuculline was not present, sIPSCs were seen in all neurons examined. Bath application of 1 \mu M nicotine increased the fre-
frequency of sIPSCs recorded from a subset (3 of 18, or 17%) of SNc DA neurons (Fig. 3, A and B). Analysis of sIPSC amplitude illustrates a pronounced nicotine-induced increase (Fig. 3C). The data from responsive and nonresponsive neurons were normalized to baseline to generate average frequency histograms for each group (Fig. 3, D and E). The responsive neurons showed an average change in sIPSC frequency similar to that observed for DA neurons in the VTA (Fig. 3F).

**Fig. 2.** Nicotine enhances glutamatergic inputs to a subset of SNc DA neurons. A: current traces showing spontaneous excitatory postsynaptic currents (sEPSCs) in the presence and absence of 1 μM nicotine. Scale bar: 50 pA, 200 ms, V_m = −70 mV. B: example frequency histogram showing the effect of 1 μM nicotine on sEPSC activity in a single SNc DA neuron (bin = 5 s). C: cumulative amplitude distribution of sEPSCs in the presence and absence of nicotine, from the cell illustrated in B. The distribution of control and nicotine amplitudes showed no significant difference (P > 0.05, Kolmogorov-Smirnov test). D: averaged normalized frequency histogram of five SNc neurons that showed an increase in sEPSC activity in response to 1 μM nicotine. E: averaged normalized frequency histogram of the 21 cells that did not show an increase in sEPSCs in response to 1 μM nicotine. F: comparison of the average increase in sEPSC frequency from the ventral tegmental area (VTA, n = 13) and SNc (n = 5). G: prevalence and pharmacology of nicotine’s effect on DA cell sEPSC activity differs in the SNc and the VTA. Cells in the SNc were tested under control conditions (n = 26), with 10 nM methyllycaconitine (MLA, n = 27), 1 μM mecamylamine (MEC, n = 14), a combination of 10 nM MLA and 1 μM MEC (n = 18), and 1 μM TTX (n = 11). Cells in the VTA were tested under control conditions (n = 21), with 10 nM MLA (n = 10), 1 μM MEC (n = 14), and 1 μM TTX (n = 3) (Mansvelder and McGehee 2000). *P < 0.05 relative to nicotine alone for each nucleus.
Although the nicotine-induced increase in sIPSC frequency in the SNc is similar to that observed in the VTA, the prevalence and pharmacology of this increase were quite different (Fig. 3G). As noted earlier, nicotine caused a significant increase in sIPSC activity in only 3/18 or 17% of DA neurons tested in the SNc; this is very different from 11/11 or 100% responsiveness in VTA DA neurons ($P < 0.0001$). As with the enhancement of sEPSC activity, neither 10 nM MLA nor 1 M nicotine caused a significant increase in sIPSC activity in response to 1 μM nicotine. F: comparison of the averaged increase in sIPSC frequency seen in the VTA ($n = 12$) and SNc ($n = 3$). G: prevalence and pharmacology of nicotine’s effect on DA cell sEPSC activity differs in the SNc and the VTA. Cells in the SNc were tested under control conditions ($n = 18$), with 10 nM MLA ($n = 15$), 1 μM MEC ($n = 12$), a combination of 50 nM MLA, 1 μM MEC, 2 μM DHβE, and 10 μM d-tubocurarine (dTC) ($n = 18$), and 1 μM TTX ($n = 19$). Cells in the VTA were tested under control conditions ($n = 12$), with 10 nM MLA ($n = 5$), 1 μM MEC ($n = 6$), and 1 μM TTX ($n = 9$) (Mansvelder et al. 2002). *$P < 0.05$ relative to nicotine alone for each nucleus.
MEC inhibited nicotine’s effects on inhibitory transmission, and rather unexpectedly, combined application of the two antagonists also failed to inhibit the response (data not shown). A combination of nAChR antagonists (2 μM DHβE, 10 μM MEC, 10 nM MLA, and 10 μM d-tubocurarine) was then employed to guarantee prevention of sIPSC enhancement if nicotinic receptors were involved, and this combination did block the effects of nicotine. This unusual pharmacology suggests that more than one subtype of nicotinic receptor mediates the enhancement of sIPSC frequency by nicotine. This may reflect the expression of the β3 subunit, expressed in GABAergic cells of the SNc and absent in similar cells in the VTA (Klink et al. 2001). In the VTA, the effect of nicotine on sIPSC activity was completely blocked by MEC, but unaffected by MLA, indicating that non-α7 receptors were responsible for that effect.

Although the nicotine-induced shift to larger sIPSC amplitudes suggests an increase in action potential activity in the GABA neurons, application of TTX (1 μM) did not completely eliminate the response to nicotine (1/19; Fig. 3G). This again contrasts with the VTA where TTX eliminated the enhancement of sIPSC frequency by nicotine. Thus nAChR expression on the GABA neurons that innervate SNc may not be exclusively segregated to somatodendritic regions and may also be expressed on presynaptic terminals.

Nicotinic responses in VTA and SNc neurons

Nicotine has been reported to induce significantly larger somatic responses from DA neurons in the VTA than in the SNc (Klink et al. 2001; Wooltorton et al. 2003). Using ACh as a nonspecific nAChR agonist and choline as a selective activator of α7 nAChRs, we assessed the relative contribution of nAChR subtypes to the responses of DA neurons in VTA and SNc. Figure 4A shows a typical response to repeated application of ACh and choline at one minute intervals. The average response to 1 mM ACh was larger for VTA than SNc DA neurons (P < 0.05; Fig. 4, B and D). Choline responses were significantly greater in VTA than in SNc on average (Fig. 4, C and D), which implies more α7 expression by VTA DA neurons, which agrees with reports from other investigators (Klink et al. 2001; Wooltorton et al. 2003). We noted a higher degree of variance in the responses of VTA neurons, and this parameter was also significantly different between the two nuclei (P < 0.05; see error bars Fig. 4D). Again, the variance of the responses was significantly greater in VTA than SNc (Fig. 4, B–D). Interestingly, the average ACh/choline response ratios were not significantly different between the two nuclei (15.38 ± 4.92 in the SNc vs. 15.04 ± 6.30 in the VTA), which suggests that stronger nicotinic responses in VTA relative to SNc may be due to differential expression of multiple receptor subtypes.
DISCUSSION

Previous physiological investigations demonstrated that systemic nicotine administration in vivo has a much more profound effect on the firing rate of DA neurons in VTA than in SNc (Mereu et al. 1987). Part of this difference may be due to different levels of somatodendritic nAChRs, as shown here and in previous studies with focal application of nicotinic agonists (Fig. 4)(Klink et al. 2001; Wooltorton et al. 2003). In addition, our data demonstrate a marked difference in functional nAChR expression on the afferent inputs to each of these nuclei. A greater prevalence of modulation of both excitatory and inhibitory synaptic inputs to the VTA by nicotine may contribute to the more profound effects of the drug on VTA dopamine neuron excitability. Both nuclei express a similar compliment of nAChR subunits (Klink et al. 2001; Wada et al. 1989). In the responsive neurons, we saw a similar magnitude of nAChR modulation of both excitatory and inhibitory synaptic inputs to VTA and SNc, but the prevalence was much lower in SNc. This suggests a different pattern of expression of nAChR in SNc, which was associated with differences in the pharmacological sensitivity of the different responses. In VTA, our evidence suggests that α4β2 nAChRs are expressed by GABA afferents and α7 nAChRs by glutamate inputs. In SNc, we found a diversity of functional receptor expression on both GABA and glutamate afferents, where combinations of antagonists were required to eliminate the responses to nicotine.

Differences in the prevalence of nicotinic modulation of synaptic inputs to SNc and VTA likely contribute to the observation that in vivo nicotine administration elicits stronger DA release in the SNc than in striatum (Di Chiara and Imperato 1988). The differences in nAChR subtype expression may also contribute to the differences in DA release. Physiological nicotine concentrations do not desensitize the α7 nAChRs on glutamate terminals to the same degree as the non-α7 subtypes (Mansvelder et al. 2002; Pidoplichko et al. 1997; Wooltorton et al. 2003). In the SNc, a combination of nAChR subtypes contribute to the nicotinic enhancement of glutamate release, as indicated by the combination of nAChR antagonists required to inhibit this effect. The non-α7 nAChRs that contribute to this effect in SNc would be expected to desensitize at nicotine concentrations relevant to tobacco use, thus limiting the impact of nicotine, and endogenous acetylcholine, on excitatory drive to the dopamine neurons. Thus the expression of different receptor subtypes, combined with lower prevalence on glutamatergic afferents, would result in a weaker response to nicotine with a much shorter duration.

We showed previously that nicotine first enhances and then suppresses inhibitory synaptic inputs to VTA DA neurons (Mansvelder et al. 2002). This occurs first through the activation of non-α7 nAChRs on the presynaptic GABA neurons leading to increased firing. This manifests as an increase in sIPSC frequency, with a corresponding increase in sIPSC amplitude due to a greater contribution of multi-vesicular synaptic events. The continued presence of nicotine ultimately desensitizes the nAChRs on the GABA neurons that underlie this effect. Normally, these receptors receive excitatory cholinergic inputs that help set the basal firing rate of the GABA neurons. Desensitization of those receptors leads to a decrease in basal GABAergic input, effectively disinhibiting the VTA DA neurons. As we saw with sEPSCs, the effect of nicotine on sIPSCs in the SNc is mediated by a mix of α7 and non-α7 nAChRs. This is demonstrated by the need for a combination of nAChR antagonists to block nicotinic potentiation of sIPSC activity. There was no evidence of a disinhibitory effect of nicotine in the SNc, which likely reflects weaker cholinergic control of the presynaptic GABA neurons that innervate these cells. The much lower prevalence of cells that showed enhanced inhibitory drive in response to nicotine (17 vs. 100% in SNc vs. VTA) also emphasizes the diminished role of nAChRs in the physiology of these GABAergic inputs.

The use of multiple antagonists to block nicotinic modulation of GABA transmission may indicate a difference in the nAChRs underlying this effect relative to the modulation of glutamate release. At this point, the most reasonable interpretation is that multiple subtypes of nAChRs mediate this effect as well as the modulation of glutamate transmission in SNc. This contrasts with the pharmacological investigations of these effects in VTA that are mediated by one nAChR subtype predominantly. The low prevalence of the responses in the SNc present a challenge for further investigations, which may require different assays.

As spontaneous synaptic currents were observed in every recorded cell, we contend that every dopamine neuron in the SNc and VTA receive both glutamatergic and GABAergic input. We do not know whether those neurons showing nicotinic modulation of glutamatergic drive are the same ones the GABAergic inputs of which are sensitive to nicotine. The prevalence of the two response types are very similar, but our experimental technique cannot monitor both postsynaptic currents at the same time. We therefore were unable to determine if nicotinic modulation of excitatory and inhibitory input occurs in the same neurons. In the VTA, the high prevalence of GABAergic modulation suggests that increased excitatory drive will be offset, at least transiently, by enhanced inhibitory input (Mansvelder et al. 2002). Presynaptic modulation of excitation and inhibition in nonoverlapping populations of DA neurons could have a much more profound effect on dopamine output from a subset of SNc neurons.

Nicotinic receptors on the DA neurons themselves are also important determinants of DA release. Consistent with the in vivo observation that nicotine induces stronger DA release from VTA, relative to SNc (Di Chiara and Imperato 1988), we found stronger responses to both ACh and choline in VTA relative to SNc. Two previous investigations have explored differences in somatic nAChR responses in VTA and SNc using similar techniques (Klink et al. 2001; Wooltorton et al. 2003). Klink et al. (2001) reported smaller somatic responses to both ACh and nicotine in SNc relative to VTA, although only the responses to nicotine were significantly different. Wooltorton et al. (2003) also reported smaller ACh responses in SNc relative to VTA but present compelling evidence that this is solely due to a difference in α7 nAChR expression in both rats and mice. We found smaller responses to choline in SNc; this supports the idea that there are differences in α7 receptor expression between these nuclei. The lack of a difference in the ratio of ACh/choline responses between the nuclei suggests that there may be differential expression of multiple nAChR subtypes. Interpretation of these findings are complicated somewhat by the observation that choline is a partial agonist of other nAChRs (Pereira et al. 2002). The large difference in ACh and choline currents (~15-fold), however,
suggest that choline only minimally activates other receptor classes in these neurons. Given the evidence that ACh and choline are equally efficacious activators of rat α7 nAChRs (Virginio et al. 2002), our data suggest that the SNc expresses lower levels of non-α7 receptors as well.

We also found a significant increase in variance of the somatic responses to ACh and choline in VTA relative to SNc. There is evidence for the existence of subpopulations of DA neurons in the VTA and SNc (Neuhoff et al. 2002; Sarabi et al. 2003; Seroogy et al. 1988), and it is possible that nicotine sensitivity may correlate with specific subpopulations. Previous investigations of ACh sensitivity in these nuclei also reported greater variance of response magnitudes in VTA relative to SNc (Wooltorton et al. 2003). Thus higher nAChR expression in a subpopulation of VTA neurons could have a profound impact on local DA levels at the sites of projection of those cells.

Our observation that nicotine can increase the excitatory and inhibitory drive to the dopaminergic cells to the SNc contradicts a previous study indicating that nicotine did not affect these inputs (Grillner et al. 2000). This is likely due to procedural differences between the two studies. Grillner et al. (2000) treated the cells with 4-AP to increase the frequency of sEPSCs, which may have masked the increase brought on by nicotine. Additionally, in their analyses of excitatory and inhibitory modulation, they tested a wide variety of conditions (1–30 μM nicotine concentration, with both focal and bath administration, n = 11), which necessarily lead to a low n for each condition. Thus their assessment of responsiveness could easily have missed an effect on a small fraction of responsive cells tested under permissive conditions. It is well known that nicotine has a classic inverted-U dose-response curve in many physiological studies including synaptic modulation (Mansvelder et al. 2002; McGeehee et al. 1995; Picciotto 2003; Pidoplichko et al. 1997; Robinson et al. 2007). Thus we would predict that the nicotine concentrations ≥10 μM employed in the study by Grillner et al. (2000) may have desensitized receptors before inducing appreciable modulation of synaptic inputs. Overall, both their study and ours support the idea that the impact of nicotine on synaptic inputs to dopamine cells is much weaker in the SNc than in the VTA.

It should also be noted that the neonatal rats used in our study may have higher expression of nAChR subunits than the adolescent animals studied by Grillner et al. (2000). Developmental expression of several nAChR subunits peak soon after birth and then decline into adulthood (Zhang et al. 1998). It may be possible that even lower prevalence and magnitude effects of nicotine would be seen in adult rats relative to the VTA. Extending these studies to older animals is another important area for future investigation.

We have shown that although nicotine causes an increase in sEPSC and sIPSC in both the SNc and the VTA, the different arrangement of nAChR subtypes that underlie these increases may result in very different physiological and behavioral outcomes. Our previous studies showed that the nAChRs that modulate synaptic inputs to the VTA are ideally situated to increase DA neuron activity, even after the nicotine has been cleared from the system (Mansvelder and McGeehee 2000; Mansvelder et al. 2002). At physiological nicotine levels, the predominantly non-α7 nAChRs on GABA neurons are desensitized, which leads to a disinhibition of the dopamine neurons

as cholinergic drive to the GABA system is blocked (Mansvelder et al. 2002). In this study, the GABA inputs to SNc were modulated by a combination of α7 and non-α7 nAChRs, which may explain why we saw no evidence of a suppression of GABA inputs after nicotine exposure—an effect that was seen in ~75% of VTA recordings (Mansvelder et al. 2002). At the same nicotine concentrations, the α7 nAChRs on glutamate terminals are not desensitized, can promote excitatory input to the DA neurons, and may even contribute to long-term potentiation of these inputs (Mansvelder and McGeehee 2000; Mansvelder et al. 2002). In the SNc, the receptors that modulate glutamate inputs also had a mixed pharmacology, suggesting that this effect would significantly desensitize at physiological nicotine levels. Thus the expression pattern of nAChRs in the SNc provides a means for nicotine-induced increases in DA neuron activity, but the lower prevalence and mixed receptor subtypes may also explain why the effects are not as profound or long-lasting.

Voltammetric studies in tissue slices have tested the effects of GABA_A, and glutamate receptor antagonists to modulate evoked somatodendritic dopamine release from VTA and SNc (Chen and Rice 2002). The findings suggest glutamatergic input to SNc acts via inhibitory inputs to decrease DA release, whereas glutamatergic innervation in VTA appeared to be more directly affecting excitability of the DA neurons. These findings may provide mechanistic insight into the differences in nicotinic modulation of at least the excitatory inputs to these two nuclei. If the density of glutamatergic innervation of DA neurons is greater in VTA than SNc, then this could explain the higher prevalence of nicotinic modulation in VTA. Additional investigations of the microcircuitry will be required to further elucidate the mechanisms that control the output of these two dopaminergic centers.

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