# Frequency-dependent modulation of dopamine release by nicotine

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**Although nicotine activation of dopamine release is implicated in addiction, it also desensitizes nicotinic acetylcholine receptors (nAChRs), leading to a prolonged depression of evoked dopamine release. Here we show that nicotine's effects depend on the firing pattern of dopamine neurons, so that while desensitization of nAChRs indeed curbs dopamine released by stimuli emulating tonic firing, it allows a rapid rise in dopamine from stimuli emulating phasic firing patterns associated with incentive/salience paradigms. Nicotine may thus enhance the contrast of dopamine signals associated with behavioral cues.**

The nucleus accumbens (NAc) shell, the principle region associated with nicotine reinforcement, is densely innervated by both intrinsic tonically active interneurons that release acetylcholine and dopaminergic neurons from the ventral tegmental area (VTA). The VTA neurons fire in a low-frequency tonic mode (0.5–8 Hz), interrupted by bursts of phasic activity (~15–50 Hz in rodents and up to 100 Hz in primates)[1,2] that are matched to salient stimuli in learning and memory experimental paradigms, including lever presses used to self-administer drugs[3]. These neurons possess both cell body and presynaptic nAChRs[4]. Nicotine shifts VTA neurons from tonic to burst firing modes[5,6] and greatly enhances extracellular dopamine, as measured by microdialysis[7]. On the other hand, nicotine at levels experienced by smokers (~250–300 nM) desensitizes nAChR so rapidly that tonic ACh activation is blocked, and evoked dopamine release is potently inhibited[8,9]. The question of how nicotine both elevates extracellular dopamine and depresses evoked dopamine release remains unresolved.

To examine the effects of presynaptic nAChR on dopaminergic VTA axons in the NAc shell, we measured extracellular dopamine at subsecond resolution using cyclic voltammetry (CV) in acutely prepared mouse corticostriatal slices. A bipolar stimulating electrode was placed in the NAc shell ~150 μm from a 5 μm diameter carbon-fiber microelectrode and depolarizing currents were applied at 2 min intervals, providing a stable dopamine response for >1 h. A single pulse stimulus (1p) elicited a maximum dopamine concentration of 0.66 ± 0.11 μM ($n$ = 8; **Fig. 1a**). The release of dopamine was enhanced at higher stimulation frequencies (20 and 100 Hz) intended to emulate

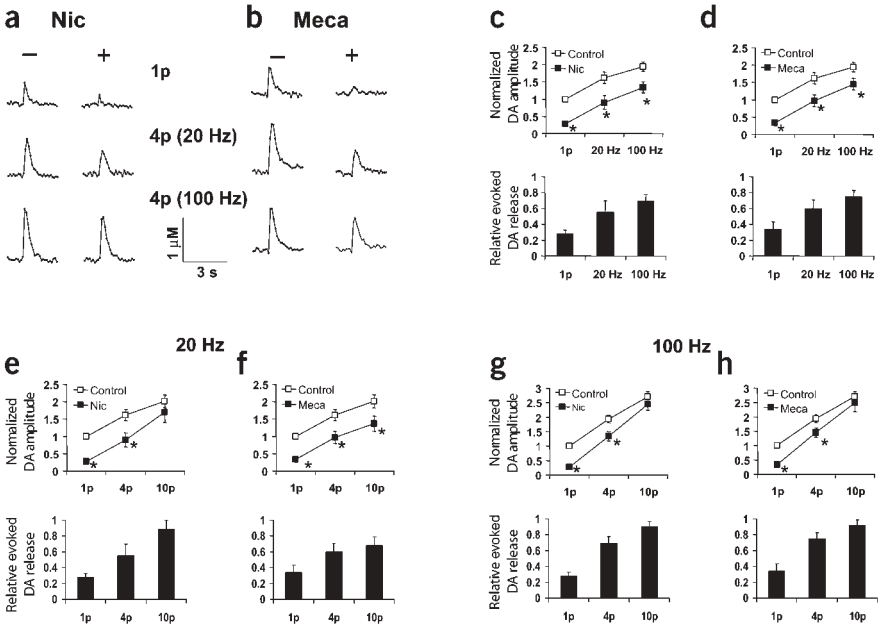


**Figure 1** Modulation of evoked dopamine (DA) release by nAChRs depends on firing pattern. (**a**,**b**) Voltammetric responses before and after 10-min bath application of nicotine (Nic, 300 nM) or mecamylamine (Meca, 2 μM) at different stimuli. The inhibition of evoked release was not blocked by D2 dopamine, GABAa, GABAb or ionotropic glutamate receptor antagonists. The slice preparation, stimulation and recording protocols were as described[15] and detailed in **Supplementary Methods** online. (**c**,**d**) Frequency modulation of nicotine and mecamylamine effects on dopamine release (mean ± s.e.m, $n$ = 8 for control; $n$ = 5 for nicotine, $n$ = 4 for mecamylamine; $^*P < 0.05$ compared with respective control values by Student's $t$-test). Top panels: evoked dopamine release normalized to that elicited by 1p stimulation under control condition. Bottom panels: relative evoked dopamine release after nicotine and mecamylamine at different stimulation frequencies. (**e**–**h**) Effects of number of pulses, nicotine and mecamylamine on dopamine release at 20 Hz (**e**,**f**) and 100 Hz (**g**,**h**) (mean ± s.e.m., $n$ = 4–8, $^*P < 0.05$ compared with respective control values by Student's $t$-test).

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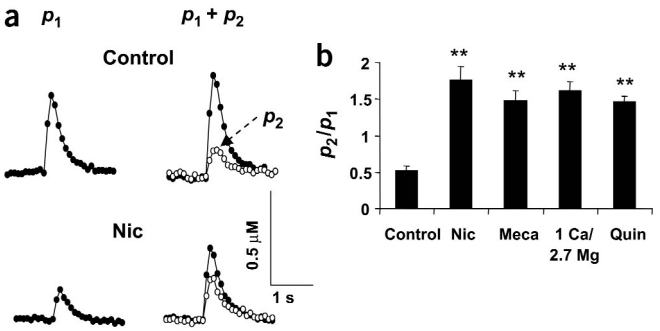


**Figure 2** Effect of nicotine on short-term facilitation. (**a**) Voltammetric responses to paired pulses at a 10 ms intervals indicate synaptic facilitation by nicotine (filled circles for the first response $p_1$ and open circles for the subtracted second response $p_2$). (**b**) Paired-pulse ratio in low concentrations of $Ca^{2+}$, nicotine, mecamylamine, and quinpirole (Quin, 0.3 µM) (mean ± s.e.m, $n = 4–8$). All interventions $^{**}P < 0.01$ by one-way ANOVA followed by Dunnett's test. The use of animals in this study followed the National Institutes of Health guidelines and was approved by the Institutional Animal Care and Use Committee of Columbia University.

phasic firing, or by increasing the number of pulses in train stimuli (**Fig. 1**).

As expected, both nicotine (Nic, 300 nM) and the nAChR antagonist mecamylamine (Meca, 2 µM) inhibited dopamine release elicited by the 1p stimulus by 72.0 ± 4.7% ($n = 8$) and 66.0 ± 9.0% ($n = 4$), respectively (**Fig. 1a–d**). The inhibition by both drugs, however, was greatly attenuated by train stimulations of 4p at 20 or 100 Hz (**Fig. 1a–d**) or when the number of pulses was increased (**Fig. 1e–h**). Notably, neither drug produced a significant effect on evoked release with 10p at 100 Hz (**Fig. 1g,h**). Thus, both blockade and desensitization of presynaptic nAChR profoundly inhibit dopamine release at low, but not high levels of presynaptic activity. These effects were not blocked by exposure to glutamate, GABA or D2 dopamine receptor antagonists (see **Supplementary Methods** online). To rule out potential effects of stimulated acetylcholine release from local cholinergic striatal interneurons, we stimulated VTA axonal fibers in the median forebrain bundle >500 µm from the recording site. The effects of nicotine were nearly identical to that of local striatal stimulation **(Supplementary Fig. 1)**.

Presynaptic nAChRs activate $Ca^{2+}$ conductances[10]. The activity dependence of desensitization-induced inhibition of dopamine release could stem from $Ca^{2+}$ effects on the probability of neurotransmitter release ($p_r$). Short-term facilitation is thought to occur when residual $Ca^{2+}$ contributes to synaptic vesicle fusion, and interventions that decrease $p_r$ produce a higher paired-pulse ratio of neurotransmitter release ($p_2/p_1$)[11]. We observed a paired-pulse depression of dopamine release under control conditions ($p_2/p_1 = 0.48 \pm 0.11$; **Fig. 2a**). Both nicotine and mecamylamine reversed the depression to elicit short-term facilitation ($p_2/p_1 = 1.8 \pm 0.2$ and $1.5 \pm 0.1$ respectively; **Fig. 2a,b**). Consistent with a reduced $p_r$ following nAChR desensitization, decreasing extracellular $Ca^{2+}$ (from 2.4 mM $Ca^{2+}$/1.3 mM $Mg^{2+}$ to 1.2 mM $Ca^{2+}$/2.7 mM $Mg^{2+}$) similarly augmented $p_2/p_1$ (**Fig. 2b**), as did the D2 dopamine receptor agonist quinpirole, which inhibits evoked dopamine release by acting on a presynaptic autoreceptor[12]. Thus, whereas nicotine-induced desensitization decreases $p_r$ and inhibits dopamine release at low activity, the inhibition is compensated during burst firing by short-term facilitation. As nicotine shifts VTA neurons from tonic to burst firing modes[5,6], the drug would be expected to induce short-term facilitation and thus elevate dopamine, as measured by microdialysis, even in the face of presynaptic receptor desensitization. Our results also suggest a reason why infusion of nicotinic antagonists into the VTA inhibits nicotine-induced dopamine release in the accumbens[13]: the antagonists block the shift from tonic to burst firing modes, as well as the associated presynaptic short-term facilitation.

Activation of the mesolimbic dopamine system is central to associative learning, reinforcement and drug addiction. As nicotine preferentially filters the dopamine signal resulting from lower tonic activity, it may enhance the dopamine response due to salient stimuli that evoke burst firing[2]. Such contrast enhancement between phasic and tonic activity may underlie nicotine enhancement of sensory gating and attention in healthy individuals and in those affected by Alzheimer disease and attention disorders[14]. Unregulated striatal dopamine is thought to underlie delusions and disorganized behavior, and it may be that contrast enhancement by nicotine underlies the very high incidence of smoking in schizophrenia, which is often suggested to be a form of self-medication.

*Note: Supplementary information is available on the Nature Neuroscience website.*

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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