Nicotine amplifies reward-related dopamine signals in striatum

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Reward-seeking behaviors depend critically on dopamine signaling—dopamine neurons encode reward-related information by switching from tonic to phasic (burst-like) activity. Using guinea pig brain slices, we show that nicotine, like cocaine and amphetamine, acts directly in striatum where it enhances dopamine release during phasic but not tonic activity. This amplification provides a mechanism for nicotine facilitation of reward-related dopamine signals, including responses to other primary reinforcers that govern nicotine dependence in smokers.

Reward-related signaling for both primary reinforcers and conditioned stimuli[1,2] is conveyed by mesostriatal dopamine release by phasic bursts of action potentials[1], rather than single-spike or tonic activity. A long-held view of nicotine addiction in tobacco smokers is that the reinforcing properties of nicotine are mediated by increased dopamine in striatum[3]. Yet paradoxically, nicotine, at concentrations achieved by smokers, desensitizes nicotinic acetylcholine receptors (nAChRs) on dopamine neurons and axons[4,5] and suppresses striatal dopamine release evoked by single action potentials[5]. Importantly, dopamine release probability in striatum is not static; it varies dynamically during a burst of action potentials[6]. Thus, nicotine reinforcement mechanisms will depend critically on the effects of nAChR desensitization on dopamine release probability during specific reward-related phasic activity.

We determined the effects of receptor-desensitizing levels of nicotine[5] on striatal dopamine release during reward-related patterns of dopamine pathway activity compared to non-reward activity. Axon terminal dopamine release was monitored in real time in slices of guinea pig dorsal striatum and nucleus accumbens using fast-scan cyclic voltammetry at carbon-fiber microelectrodes[6] with a sampling frequency of 8–10 Hz (see **Supplementary Methods** online). Slices were prepared according to Home Office (UK) guidelines. Stimulation was local[6] using a perimaximal current (200 μs duration) optimized for single-pulse evoked release (**Supplementary Methods**). Stimulation frequencies represented the physiological range of dopamine neuron firing frequencies[7,8]—tonic (5–10 Hz) and phasic burst-like (20–100 Hz)—that typically accompany presentation of a reinforcer or reward-predictor[7]. Consistent with facilitation of initial dopamine release by endogenous ACh and the expected nAChR desensitization by nicotine[5], striatal dopamine release evoked by single pulses was reversibly diminished by nicotine (500 nM) or the nAChR antagonist mecamylamine (**Fig. 1a,b**). During five-pulse trains in control, peak extracellular dopamine concentration ($[DA]_o$) was relatively insensitive to frequency (**Fig. 1**). However, nicotine, like the nAChR antagonist mecamylamine, suppressed release elicited by single pulses or low, tonic frequencies (≤10 Hz), but selectively enhanced release by phasic bursts (≥25 Hz; **Fig. 1a–c**). Thus nicotine, acting by nAChR desensitization, enhanced the contrast between phasic- and tonic-evoked $[DA]_o$ (**Fig. 1d**).

To understand the dual action of nicotine—enhancing phasic release while suppressing initial (and tonic) release—we examined the effect of nicotine on the dynamic release probability of dopamine (relative release per pulse) within a phasic burst (100 Hz). Under control conditions, there is a negligible 'gain' on release evoked by multiple versus single pulses: short-term depression of release rapidly occurs at striatal synapses[6] and diminishes release probability at successive pulses[6] (**Fig. 2**). In contrast, nicotinic suppression of initial pulse-evoked

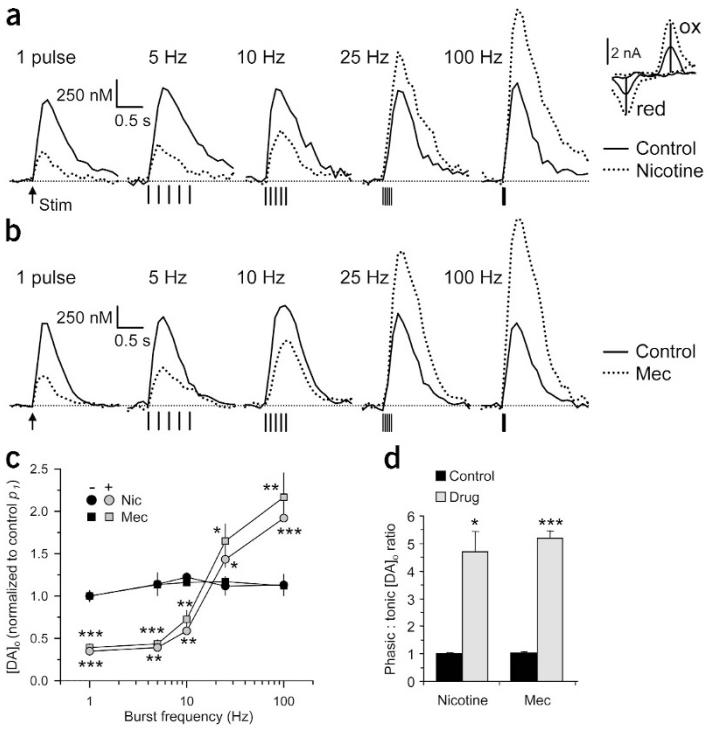

Figure 1 Nicotine enhances the frequency-sensitivity of dopamine release and phasic-versus-tonic contrast. (**a**,**b**) Averaged profiles of $[DA]_o$ versus time at a typical site in control and in (**a**) nicotine ($n = 3–6$) or (**b**) mecamylamine (Mec; 20 μM; $n = 2–11$). Inset, typical cyclic voltammograms elicited at 100 Hz; dopamine was identified by characteristic oxidation (ox) and reduction (red) peak potentials (+600 and –200 mV vs. Ag/AgCl). (**c**) Mean peak $[DA]_o \pm$ s.e.m. versus frequency in controls, nicotine ($n = 3–11$) or mecamylamine ($n = 17–19$), normalized to control p_1. Control (–); with drug (+). (**d**) Phasic:tonic ratios of mean peak $[DA]_o$ at 100 Hz versus 5 Hz (5-pulse trains, $n = 3–10$). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (compared to control).

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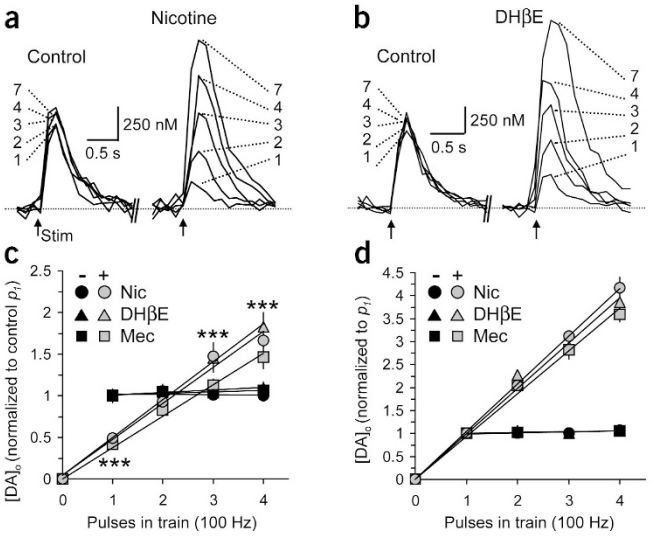

Figure 2 Nicotine gates dopamine release probability to increase release during burst-like activity. (**a**,**b**) Averaged profiles of $[DA]_o$ following 1–7 pulses (arrows) at 100 Hz for typical sites in control (left) versus (**a**) nicotine (500 nM) or (**b**) DHβE (100 nM) (right) (n = 2–5). For higher pulse numbers, see **Supplementary Note** online. (**c**,**d**) Mean peak $[DA]_o$ ± s.e.m. versus number of pulses per burst in control, nicotine (n = 5–46), mecamylamine (n = 5–23) or DHβE (n = 5–26) normalized to (**c**) control p_1 or (**d**) drug p_1. After nAChR desensitization/blockade, $[DA]_o$ was proportional to pulse number, which (**c**) generated a larger range of $[DA]_o$ (***$P < 0.001$ vs. control) and (**d**) changed the slope, or 'gain', to unity (0.93–1.0; $R^2 > 0.99$). Control (–); with drug (+).

release (**Figs. 1a** and **2**) was accompanied by relief of short-term depression: release was equivalent per successive pulse in the burst (**Fig. 2**). These effects were mimicked by mecamylamine or dihydro-β-erythroidine (DHβE; **Fig. 2b**,**c**), a selective antagonist of nAChRs containing the β2 subunit (β2*nAChRs), which are those expressed on dopamine axons[9,10]. Thus β2*nAChRs normally gate the dynamic probability of dopamine release. By reorganizing release probability, nicotine relieves short-term depression during phasic bursts.

We explored the frequency-specific effect of nicotine (**Fig. 1**) by determining the interaction between firing frequency and dynamic release probability using paired pulses at varying inter-pulse intervals. Paired-pulse release ratios (p_2/p_1) showed characteristic short-term depression in controls[6] at all inter-pulse intervals (**Fig. 3**). However, following nAChR desensitization/blockade (nicotine, mecamylamine or DHβE), a change in frequency filtering[11] occurred at >12.5 Hz (80 ms interval): p_2/p_1 increased with shorter inter-pulse intervals, resulting in greater relief of depression at higher frequencies (**Fig. 3**). Nicotine, in effect, sets a high-frequency pass filter[11] for impulse-dependent dopamine release.

Thus we show that nicotine, through nAChR desensitization, suppresses dopamine release during non-reward, low firing frequencies, and conversely, selectively enhances reward-related dopamine release by relieving short-term depression at higher burst-like frequencies. The findings suggest that nicotine will enhance the contrast in $[DA]_o$ when dopamine neuron activity switches from tonic to phasic firing, which could be in response to salient primary rewards or conditioned reward predictions[7]. This dependence on dopamine neuron firing rate reconciles previously conflicting data on striatal nicotine effects[3,5] and explains why local actions in striatum have been underestimated[12]. Moreover, these striatal effects provide a neurochemical correlate for the nicotine enhancement of the reinforcing efficacy of any reward-related stimuli, including non-nicotine conditioned stimuli (*e.g.*, visual cues) that may support nicotine self-administration[13]. In combination with the actions of nicotine on somatodendritic excitability[4,14,15], these direct effects on striatal axons will exaggerate dopaminergic mechanisms of reinforcement to provide a powerful teaching signal[1] for nicotine-associated learned behaviors, habits and addiction at the synaptic level[2].

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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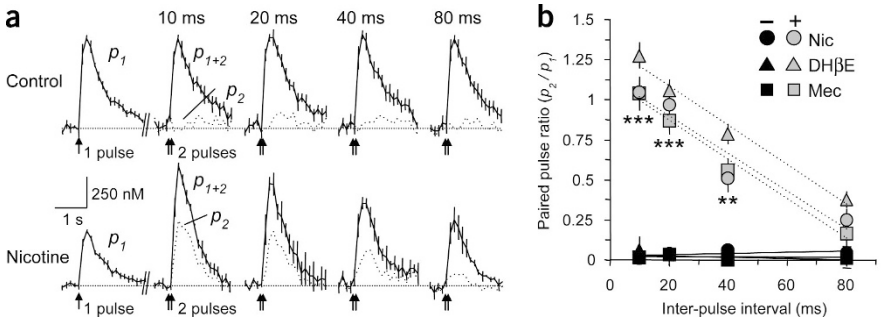

Figure 3 Nicotine in striatum switches the 'frequency filtering' of dopamine release. (**a**) Profiles of $[DA]_o$ ± s.e.m. elicited by 1 (p_1) or 2 pulses (p_{1+2}) paired at inter-pulse intervals equivalent to 100–12.5 Hz in control (top row) versus nicotine (500 nM; n = 3–11; bottom row) at a typical site; p_2 is p_{1+2} minus p_1. (**b**) Average paired-pulse ratios (p_2/p_1). Control: slope not different from zero ($P > 0.05$, R^2 = 0.05–0.31); nAChR desensitization/blockade: linear inverse dependence of p_2/p_1 on pulse interval (dotted; slope vs. zero: $P < 0.01$–0.05; $R^2 > 0.92$; n = 3–20). **$P < 0.01$, ***$P < 0.001$ (versus control). Control (–); with drug (+). Note, amplification at high frequencies failed at inter-pulse intervals <10 ms (see **Supplementary Note** and **Supplementary Fig. 1** online).

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