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α6-Containing Nicotinic Acetylcholine Receptors Dominate the Nicotine Control of Dopamine Neurotransmission in Nucleus Accumbens

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Modulation of striatal dopamine (DA) neurotransmission plays a fundamental role in the reinforcing and ultimately addictive effects of nicotine. Nicotine, by desensitizing β2 subunit-containing (β2*) nicotinic acetylcholine receptors (nAChRs) on striatal DA axons, significantly enhances how DA is released by reward-related burst activity compared to nonreward-related tonic activity. This action provides a synaptic mechanism for nicotine to facilitate the DA-dependent reinforcement. The subfamily of β2*-nAChRs responsible for these potent synaptic effects could offer a molecular target for therapeutic strategies in nicotine addiction. We explored the role of α6β2*-nAChRs in the nucleus accumbens (NAc) and caudate-putamen (CPu) by observing action potential-dependent DA release from synapses in real-time using fast-scan cyclic voltammetry at carbon-fiber microelectrodes in mouse striatal slices. The α6-specific antagonist α-conotoxin-MII suppressed DA release evoked by single and low-frequency action potentials and concurrently enhanced release by high-frequency bursts in a manner similar to the β2*-selective antagonist dihydro-β-erythroidine (DHβE) in NAc, but less so in CPu. The greater role for α6*-nAChRs in NAc was not due to any confounding regional difference in ACh tone since elevated ACh levels (after the acetylcholinesterase inhibitor ambenonium) had similar outcomes in NAc and CPu. Rather, there appear to be underlying differences in nAChR subtype function in NAc and CPu. In summary, we reveal that α6β2*-nAChRs dominate the effects of nicotine on DA release in NAc, whereas in CPu their role is minor alongside other β2*-nAChRs (eg α4*), These data offer new insights to suggest striatal α6*-nAChRs as a molecular target for a therapeutic strategy for nicotine addiction.
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INTRODUCTION

Striatal dopamine (DA) neurotransmission plays key roles in signaling information about natural as well as artificial reinforcers like addictive drugs (including nicotine) (Corrigall *et al*, 1992; Imperato *et al*, 1986; Nisell *et al*, 1994; Schultz, 2002; Wise, 2004). In particular, mesostriatal dopaminergic neurons signal reward-predicting stimuli and receipt of unpredicted rewards by brief bursts of high-frequency neuron activity (Schultz, 1986, 2002). Striatal nicotinic acetylcholine receptors (nAChRs) operate a powerful and complex neuromodulatory control over the dynamic probability of DA release during such bursts as well as non-burst activity (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Zhou *et al*, 2001). Tonic levels of striatal ACh at β2-subunit-containing (β2*)-nAChRs promote DA release by individual action potentials but, owing to accompanying short-term synaptic depression, minimize the release of DA by subsequent action potentials in high-frequency bursts (Cragg, 2006; Rice and Cragg, 2004). Nicotine, at concentrations seen in smokers, desensitizes tonically active β2*-nAChRs (Mansvelder *et al*, 2002; Pidoplichko *et al*, 1997); in striatum, this reduces initial DA release probability, removes short-term depression and consequently facilitates release by bursts (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Zhou *et al*, 2001).

Nicotine also has effects at the somatodendritic level on DA neurons and inputs. Through a complex activation and desensitization of α7 and β2*-nAChRs respectively in midbrain, nicotine increases DA neuron excitability (see Grenhoff *et al*, 1986; Mansvelder *et al*, 2002; Schilstrom *et al*, 2003). Together these somatodendritic and axonal actions offer a two-step mechanism (Exley and Cragg, in press) through which nicotine can promote how burst activity in DA neurons facilitates DA-dependent reinforcement processing that ultimately contributes to addiction.

The neurochemical as well as corresponding reinforcing effects of nicotine depend critically on β2*-nAChRs (Picciotto *et al*, 1998). However, β2*-nAChRs comprise a

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diverse family expressed widely throughout the brain. The identity of the corresponding α-subunits of the specific nAChRs that participate in the effects of nicotine within striatum are unresolved. Rodent striatal DA axon terminals contain β2 subunits coexpressed predominantly with α4, or α6, α5 and also β3 subunits within at least three types of heteromeric pentamers, including α4β2, α6β2β3, and α6α4β2β3 (Champtiaux *et al*, 2003; Charpantier *et al*, 1998; Cui *et al*, 2003; Exley and Cragg, in press; Grady *et al*, 2002; Klink *et al*, 2001; Quik and McIntosh, 2006; Quik *et al*, 2005; Salminen *et al*, 2004; Zoli *et al*, 2002). Unlike other subunits, expression of the α6 subunit is relatively restricted to catecholaminergic (and some visual system) neurons (Le Novere *et al*, 1996; Quik *et al*, 2001, 2002). Moreover, after somatic expression of α6 mRNA in VTA/SN DA neurons (Azam *et al*, 2002, 2007), plasmamembrane α6*-nAChRs on DA axon terminals (Quik *et al*, 2002; Zoli *et al*, 2002) may account for up to 40% of β2*-nAChRs (in rat) (Zoli *et al*, 2002). Given their restricted localization to striatal DA axons, α6*-nAChRs are attracting attention as promising targets for selective pharmacotherapies in dopaminergic disorders including nicotine addiction and Parkinson's disease (Quik and McIntosh, 2006).

Here, by detecting dopamine release in real time using fast-scan cyclic voltammetry (FCV) at carbon-fiber microelectrodes in striatal slices, we explored directly the role of striatal α6β2*-nAChRs in the dynamic control by endogenous ACh and nicotine of DA release probability during burst and nonburst activity. We reveal a role for α6β2*-nAChRs in the dynamic control of striatal DA release probability that differs markedly between the NAc and CPu to which different aspects of motivational and sensorimotor function are attributed.

MATERIALS AND METHODS

Slice Preparation and Voltammetry

Coronal striatal slices, 300 μm thick, containing both nucleus accumbens (NAc) and caudato-putamen (CPu) were prepared from brains of C57/Bl6j mice (20–30 g; Charles River UK) using previously described methods (Cragg, 2003; Rice and Cragg, 2004). Extracellular DA concentration ($[DA]_o$) was monitored at 32°C in bicarbonate-buffered artificial cerebrospinal fluid (containing 2.4 mM Ca^{2+}) using FCV with 7 μm carbon-fiber microelectrodes (tip length ~50–100 μm, fabricated in-house) and a Millar Voltammeter (PD Systems, UK) as described previously (Cragg, 2003; Rice and Cragg, 2004). In brief, the scanning voltage was a triangular waveform (−0.7 to +1.3 V range *vs* Ag/AgCl) at a scan rate of 800 V/s and sampling frequency of 8 Hz. The evoked current signal was attributed to DA by comparison of the potentials for peak oxidation and reduction currents with those of DA in calibration media (+500–600 and −200 mV *vs* Ag/AgCl, respectively). Electrodes were calibrated in 1–2 μM DA in experimental media.

Electrical Stimulation

DA release was evoked by a surface, bipolar concentric electrode (25 μm diameter Pt/Ir; FHC, USA) ~100 μm from the recording electrode (Cragg, 2003; Cragg and Greenfield, 1997; Rice and Cragg, 2004). Stimulus pulses (200 μs duration) were generated out-of-phase with FCV scans at currents (0.5–0.7 mA) that generate maximal DA release with a single pulse. Release is Ca^{2+}-dependent and TTX-sensitive (ie action potential-dependent) (Cragg, 2003; Cragg and Greenfield, 1997).

Evoked DA release on the timescale of these experiments is not modulated by glutamate or GABA at ionotropic receptors (Avshalumov *et al*, 2003; Cragg, 2003), or DA D_2 receptors (Cragg, 2003). However, DA release is governed by a basal tone of endogenous ACh acting at presynaptic nAChRs on DA axons (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Zhou *et al*, 2001). Local cholinergic interneurons in striatal slice preparations are spontaneously active as they are *in vivo* (Aosaki *et al*, 1994; Bennett and Wilson, 1999), and provide a dense network of ACh release sites (Contant *et al*, 1996; Descarries and Mechawar, 2000; Zhou *et al*, 2003) that can provide this background of endogenous ACh (Zhou *et al*, 2001). ACh release evoked by the stimulus does not appear to contribute to these effects: similar results were seen in pilot studies in parasagittal slices in which DA release was elicited by remote pathway stimulation (up to 1000 μm ventrocaudal from the recording electrode) (Rice and Cragg, 2004).

Experimental Design and Analysis

Stimuli were repeated at 3-min intervals to ensure consistent release. Stimuli consisted of either single pulses (1 pulse, p) or bursts of pulses (2–5 p) at a physiological range of DA neuron firing frequencies which included 'tonic' rates (1–10 Hz) and 'phasic' burst frequencies (25–100 Hz) that mimic the firing patterns of DA neurons that accompany 'reward-related' events *in vivo* eg the presentation of primary reward, a conditioned reinforcer or reward-predictor (Bayer and Glimcher, 2005; Hyland *et al*, 2002; Schultz, 1986). The term 'burst sensitivity' refers to the relative sensitivity of DA release to a burst *vs* a single pulse and denotes the ratio of release by a 4 p burst *vs* 1 p.

Data are means ± SEM and the sample size, *n*, is the number of observations. Number of animals in each data set is ⩾3. Comparisons for differences in means were assessed by one- or two-way ANOVA, *post hoc* multiple comparison *t*-tests (Bonferroni), or unpaired *t*-tests using GraphPad Prism. Curve fitting and linear regressions were performed in GraphPad Prism or SigmaPlot.

Recording sites were in the dorsal quartile of CPu or immediately ventral to the anterior commissure (NAc 'core'). Note that in NAc, a small population of recording sites (~20%) displayed only modest nAChR control of DA release (as revealed by the nAChR antagonist DHβE) and not the strong control seen more commonly. This observation may be consistent with immunocytochemical studies suggesting that the ventral striatum exhibits a heterogeneity of cholinergic innervation greater than in dorsal striatum (Phelps and Vaughn, 1986). Since the aim of the current study is to explore the role of the α6-subunit where nAChRs regulate DA release, data from this minority of recording sites were not included for analysis.

Drugs

α-CtxMII was synthesized as described previously (Cartier *et al*, 1996). α-CtxMII is a selective antagonist for

α3/α6*-nAChRs (Cartier *et al*, 1996; Kuryatov *et al*, 2000), but since there is negligible presence of the α3-subunit in mouse striatum (Champtiaux *et al*, 2003; Zoli *et al*, 2002), α-CtxMII is selective for α6*-nAChRs (Champtiaux *et al*, 2002; Quik and McIntosh, 2006; Whiteaker *et al*, 2000). In the striatum, α-CtxMII-sensitive α6*-nAChRs include α6β2β3 and α6α4β2β3-nAChRs (Salminen *et al*, 2004, 2005). The concentration of α-CtxMII used here (30 nM) has no reported detectable effects at non-α6/α3-nAChRs (Cartier *et al*, 1996) and maximally inhibits the α-CtxMII-sensitive component of nicotine-evoked [^{3}H]-DA release from striatal synaptosomes (Grady *et al*, 2002; McCallum *et al*, 2005; Salminen *et al*, 2004). Higher concentrations of α-CtxMII (100 nM) in this study had no additional effects (not illustrated). The effects of DHβE were maximal at the concentrations used in this study (100 nM–1 μM) (data not illustrated). Ambenonium dichloride and α-bungarotoxin were purchased from Tocris Bioscience (UK). All other reagents were purchased from Sigma-Aldrich (UK).

RESULTS

Nicotine Gates DA Release Probability in CPu and NAc via β2*-nAChRs

In order to elucidate the role of striatal α6*-nAChRs in the control of DA release by striatal ACh and nicotine, we first confirmed reported effects of nicotine on the dynamic properties of DA release probability in CPu and verified similar effects in NAc of the mouse. Nicotine (500 nM) desensitizes striatal nAChRs and thus reverses actions of endogenous striatal ACh (Rice and Cragg, 2004; Zhou *et al*, 2001). We used single stimulus pulses and bursts of pulses (2–4 p at 100 Hz) to explore the dynamic regulation of DA release probability during bursts. These initial experiments used this intraburst frequency (100 Hz) which is at the uppermost range of frequencies observed *in vivo,* since this frequency most strongly reveals nAChR control in CPu (Rice and Cragg, 2004).

In control conditions, there was a limited greater sensitivity of DA release to multiple *vs* single pulses in CPu or NAc as previously described (Cragg, 2003; Rice and Cragg, 2004) eg a burst of 4 p (100 Hz) evoked only slightly but significantly greater $[DA]_o$ than a single pulse in CPu (Figure 1a and b) and in NAc (Figure 1c and d). Typically, ratios of $[DA]_o$ evoked by 4 p *vs* 1 p in CPu were ~1.1-fold (~2.3 *vs* ~2.0 μM), and in NAc were ~1.3-fold (~1.2 *vs* ~1.6 μM). This limited difference in $[DA]_o$ evoked by burst *vs* nonburst stimuli is characteristic of the short-term synaptic depression which rapidly occurs at striatal DA synapses and limits re-release at successive pulses within a burst (Cragg, 2003; Rice and Cragg, 2004). Nicotine (500 nM) had significant effects on DA release probability in both CPu and NAc: $[DA]_o$ evoked by 1 p were

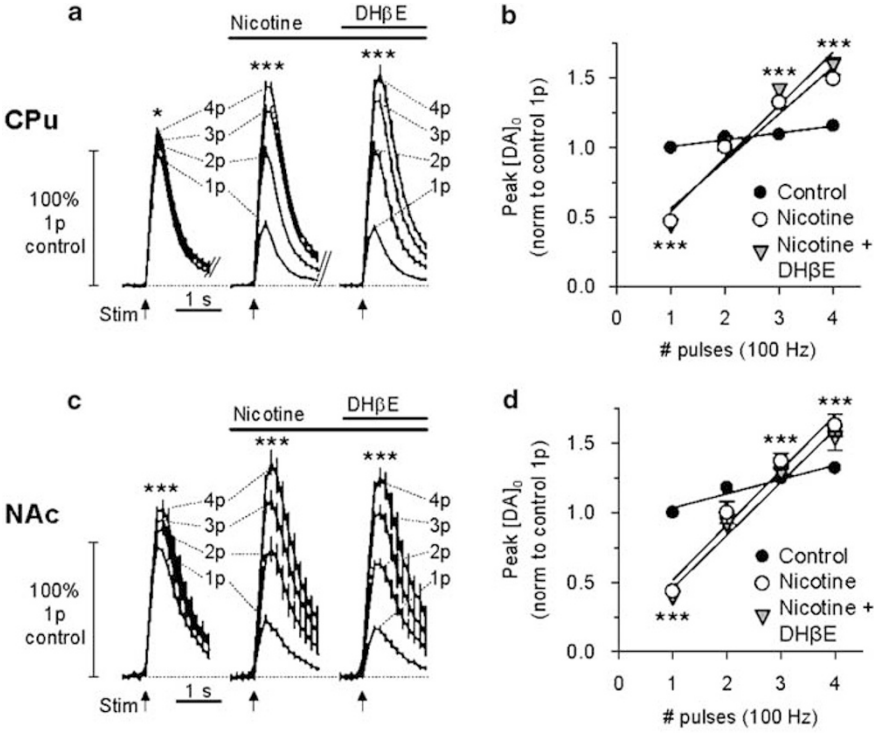

Figure 1 Nicotine gates striatal dopamine (DA) release probability in CPu and NAc via β2*-nAChRs. (a, c) Averaged profiles of $[DA]_o$ following stimuli (*arrows*) of 1–4 pulses (p) at 100 Hz in (*left*) control conditions, (*center*) nicotine (500 nM) or (*right*) nicotine plus DHβE (100 nM) normalized to $[DA]_o$ released by 1 p in controls in (a) CPu and (c) NAc. One-way ANOVA for number of pulses, $^*P<0.05$, $^{***}P<0.001$, $n=8$–43. (b, d) Mean peak $[DA]_o \pm$ SEM *vs* number of pulses in a burst (100 Hz) in controls (*filled circles*), or nicotine (*unfilled*), or nicotine plus DHβE (*triangles*) normalized to $[DA]_o$ released by 1 p in controls in (b) CPu and (d) NAc. As previously in CPu after nAChR desensitization by nicotine (or nAChR block, (Rice and Cragg, 2004)), nicotine or nicotine plus DHβE enhanced the sensitivity of DA release to pulse number (slope, or 'gain' increased to unity (0.93–1.0; $R^2>0.99$), and enhanced the range of $[DA]_o$ in both (b) CPu and (d) NAc. Comparisons of linear regressions for control *vs* nicotine: $P<0.01$, $n=8$–39. *Asterisks*: Comparisons *vs* controls, *post hoc* Bonferroni *t*-tests, $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Block of nAChRs by maximal DHβE (100 nM) was not additive upon nAChR desensitization by nicotine ($n=8$–18; *Solid lines*: linear regressions: $R^2>0.94$, nicotine not different to nicotine plus DHβE, comparison of linear regressions: $P>0.05$).

significantly reduced to ~45% in both CPu (Figure 1a and b) and NAc (Figure 1c and d). Correspondingly, the sensitivity of DA release to multiple pulses in a burst was significantly enhanced by nicotine in both CPu and NAc (Figure 1b and d) resulting in a significant enhancement compared to control of $[DA]_o$ evoked by 4 p (100 Hz) to ~140% of control in CPu (Figure 1a and b) and to ~120% in NAc (Figure 1c and d). Thus, in the presence of nicotine, ratios of $[DA]_o$ released by 4 p *vs* 1 p were enhanced to approximately 3–4 in both CPu and NAc (ie linear response to pulse number) (Figure 1a–d). These observations are similar to previously documented effects of nicotine in CPu (Rice and Cragg, 2004), where nicotine acting through nAChR desensitization reduces initial release probability at a single pulse and consequently relieves short-term depression which permits enhanced release by successive pulses within a burst.

We and others have previously shown that these effects of nicotine are indistinguishable from those of the nAChR antagonist dihydro-β-erythroidine (DHβE) indicating that nicotine is acting via desensitization of endogenous ACh tone at nAChRs (Rice and Cragg, 2004; Zhou *et al*, 2001). In the current study, subsequent coapplication DHβE (100 nM) in the continued presence of nicotine did not further modify DA release compared to nicotine alone in either CPu or NAc (Figure 1) confirming that these effects of nicotine are due to desensitization of the entire population of striatal β2*-nAChRs that were activated by striatal ACh.

Dominant Role for α6β2*-nAChRs in NAc but not CPu

To identify the role of striatal α6 subunits in the potent β2*-nAChR regulation of DA release in CPu and NAc, we used the α6-selective nAChR antagonist, α-CtxMII (Champtiaux *et al*, 2002; Nicke *et al*, 2004; Whiteaker *et al*, 2000). DHβE was subsequently coapplied to compare the effects of antagonism of the whole β2*-nAChR population upon which nicotine acts. We first explored the effects of α-CtxMII on the regulation of release by 1 or 4 p (100 Hz burst). In CPu, in control conditions, $[DA]_o$ evoked by a 4-p burst was slightly but significantly greater than release by a single pulse (Figure 2a) as seen previously (Figure 1). Application of α-CtxMII (30 nM) to CPu modestly but significantly reduced $[DA]_o$ evoked by a single pulse and slightly reduced $[DA]_o$ evoked by 4 p/100 Hz (Figure 2a and b), thus slightly but significantly increasing the sensitivity of DA release to burst *vs* nonburst stimuli compared to controls (Figure 2b). However, subsequent coapplication of DHβE to CPu in the continued presence of α-CtxMII significantly further reduced $[DA]_o$ evoked by 1 p compared to α-CtxMII and also enhanced $[DA]_o$ evoked by a 4 p/100 Hz burst compared to α-CtxMII (and compared to

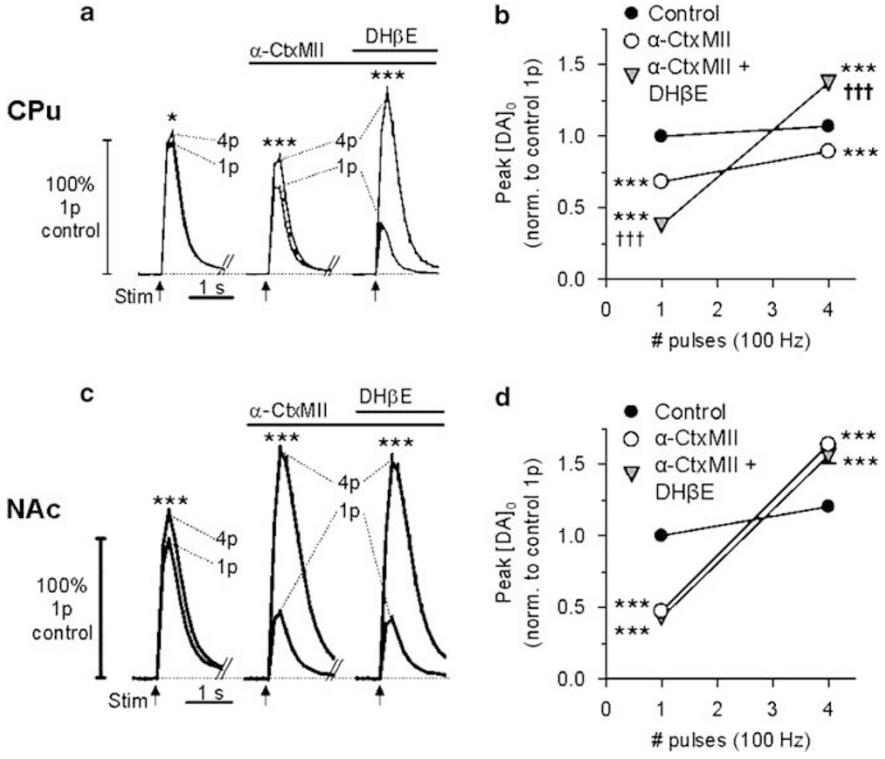

Figure 2 α-CtxMII reveals a dominant control of dopamine release by α6β2*-nAChRs in NAc but not CPu. (a, c) Averaged profiles of $[DA]_o$ following stimuli (*arrows*) of 1 or 4 pulses (p) at 100 Hz in (*left*) control conditions, (*center*) α-CtxMII (30 nM) or (*right*) α-CtxMII plus DHβE (1 μM) normalized to $[DA]_o$ released by 1 p in controls in (a) CPu and (c) NAc. One-way ANOVA for number of pulses, $^*P<0.05$, $^{***}P<0.001$, $n=18$–63. (b, d) Mean peak $[DA]_o \pm$ SEM *vs* number of pulses (1 or 4 p, 100 Hz) in controls (*filled circles*), α-CtxMII (*unfilled*), or α-CtxMII plus DHβE (*triangles*) normalized to $[DA]_o$ released by 1 p in controls in (b) CPu and (d) NAc. Significance of *post hoc t*-tests indicated by asterisks for comparisons *vs* controls ($^*P<0.05$, $^{***}P<0.001$) and by cruciform symbols for comparisons *vs* α-CtxMII ($^{\dagger\dagger\dagger}P<0.001$). (b) In CPu, α-CtxMII reduced $[DA]_o$ evoked by 1 and 4 p with slight, significant increase in sensitivity of DA release to number of pulses while addition of DHβE further modified $[DA]_o$ and sensitivity of DA release to a burst. $n=19$–63. (d) In NAc, α-CtxMII significantly reduced 1 p release, enhanced burst release and enhanced sensitivity of DA release to number of pulses in a manner not modified further by DHβE. $n=16$–52.

drug-free controls) (Figure 2a and b). Thus, DHβE dramatically promoted the relative sensitivity of DA release to a 4-p burst *vs* a single pulse compared to α-CtxMII (Figure 2a and b).

In NAc, in control conditions, $[DA]_o$ evoked by a 4-p burst (100 Hz) was slightly but significantly greater than release by a single pulse (Figure 2c and d) as seen previously (Figure 1). Application of α-CtxMII (30 nM) to NAc significantly reduced $[DA]_o$ evoked by a single pulse and also increased $[DA]_o$ evoked by 4 p/100 Hz (Figure 2c and d) thus enhancing the sensitivity of DA release to a burst compared to controls (Figure 2d). However, subsequent coapplication of DHβE in the continued presence of α-CtxMII did not further modify release by 1 or 4 p (Figure 2c and d) or the relative sensitivity of DA release to each stimulus (Figure 2d).

In separate experiments, application of the selective α7-nAChR antagonist α-bungarotoxin alone (100 nM) did not modify $[DA]_o$ evoked by either a single pulse stimulation or a high-frequency burst (data not illustrated) indicating a lack of control of DA release by α7*-nAChRs.

α6β2*-nAChRs Can Account for All Frequency Filtering of DA Release by nAChRs in NAc but not CPu

Changes in dopaminergic firing frequency within a burst are critical to the processing of reward-related information: Typically, low-frequency activity (0.5–5 Hz) is associated with nonreward-related activity, while high frequencies (20–100 Hz) signal reward-related information (Hyland *et al*, 2002; Schultz, 1986, 2002). We have previously shown that nAChRs critically govern how DA is released by different frequencies of presynaptic activity and that nAChR desensitization by nicotine changes this frequency filtering (Rice and Cragg, 2004; Zhang and Sulzer, 2004). By reducing DA release at low-frequency firing, and enhancing DA release at high-frequency firing, nicotine enhances the contrast in DA signals released by a different DA neuron frequency.

We explored the component role of α6*-nAChRs in the regulation of DA signals evoked by reward-related frequencies *vs* nonreward-related frequencies by using 5-p trains at frequencies ranging from 1 to 100 Hz. In control conditions, $[DA]_o$ varied significantly with frequency according to a slight inverted U-relationship in both CPu (Figure 3a and b) and NAc (Figure 3c and d). In CPu, the addition of α-CtxMII slightly reduced $[DA]_o$ at all frequencies compared to control but also slightly enhanced the relationship between $[DA]_o$ and frequency (Figure 3a and b). Specifically, the ratio of $[DA]_o$ released by 100 Hz compared to a single pulse was enhanced in the presence of α-CtxMII compared to control (Figure 3b). However, subsequent coapplication of DHβE in the continued presence of α-CtxMII in CPu had additional frequency-specific effects. DHβE reduced $[DA]_o$ evoked by low stimulation frequencies ($\leqslant$25 Hz) but enhanced $[DA]_o$ evoked by higher frequencies ($>$25 Hz)

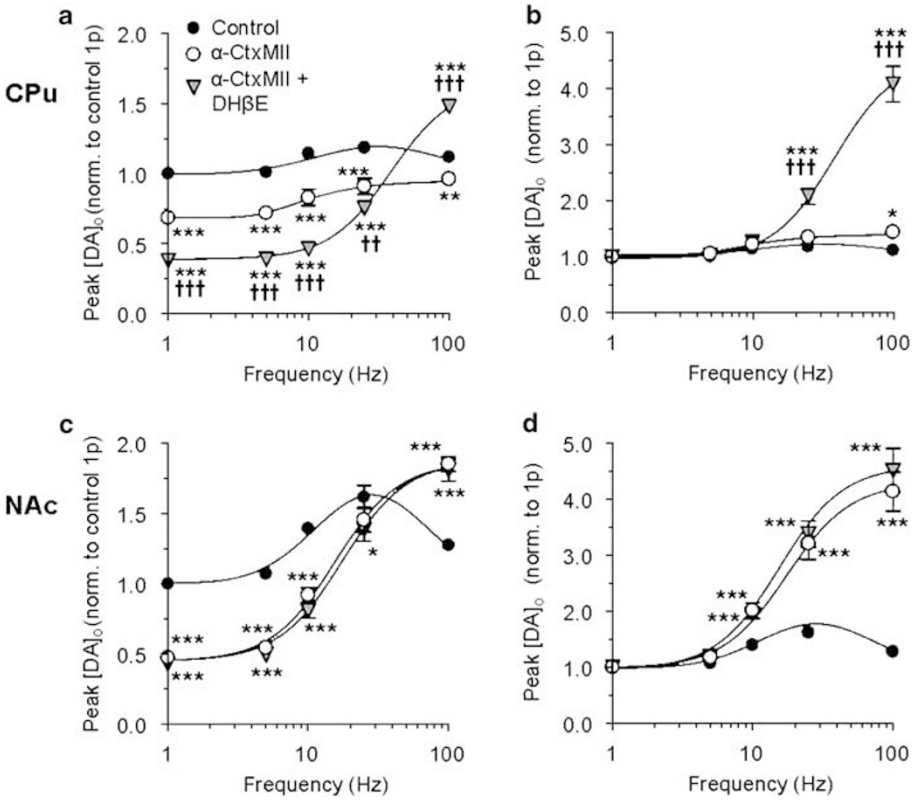

Figure 3 α6β2*-nAChRs can account for all frequency filtering of dopamine release by nAChRs in NAc but not CPu. (a–d) Mean peak $[DA]_o \pm$ SEM *vs* frequency during 5-pulse (p) trains (1–100 Hz) in controls (*filled circles*), α-CtxMII (*unfilled*), or α-CtxMII plus DHβE (*triangles*) normalized to $[DA]_o$ released by (a, c) 1 p in controls or (b, d) 1 p in each drug condition in (a, b) CPu ($n = 12$–63) and (c, d) NAc ($n = 9$–52). Significant effects of frequency: One-way ANOVAs $P < 0.001$. Curve-fits in this frequency range are modified Gaussian (controls, $R^2 > 0.9$) or sigmoidal curves ($R^2 > 0.99$). Significance of *post hoc* *t*-tests indicated by asterisks for comparisons *vs* controls (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) and by cruciform for comparisons of DHβE *vs* α-CtxMII ($^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$).

and thus enhanced the absolute concentration range (Figure 3a) as well as frequency-sensitive contrast (frequency sensitivity) of evoked $[DA]_o$ compared to α-CtxMII and controls (Figure 3b).

In NAc by contrast, the addition of α-CtxMII alone reduced $[DA]_o$ at low frequencies ($\leqslant$10 Hz) compared to controls and increased $[DA]_o$ by frequencies >25 Hz compared to controls (Figure 3c and d). Thus, α-CtxMII alone enhanced the absolute concentration range (Figure 3c) and frequency-sensitive contrast of evoked $[DA]_o$ in NAc compared to control (Figure 3d). In NAc, the addition of DHβE to α-CtxMII did not further modify $[DA]_o$ or its frequency-dependence (Figure 3c and d). Thus, selective block of α6*-nAChRs antagonizes apparently all of the frequency-specific filtering effects of β2*-nAChRs in NAc, but only a partial component in CPu.

Regional Differences in α6-nAChR Function Are Not due to Confounding Differences in Endogenous ACh Tone or DA Release Probability

These data suggest that striatal ACh and nicotine regulate DA release probability primarily via α6β2*nAChRs in NAc, and via a more mixed population including non-α6, β2*-nAChR subtypes in CPu. The greater role for α6*-nAChRs in NAc could be due to a difference in the functional nAChRs present in axon terminals in NAc and CPu. Alternatively, this functional difference could arise from an underlying similar receptor stoichiometry if there are differences in key driving forces eg ACh tone (or other factors contributing to DA release probability) in NAc and CPu. For example, if ACh tone at striatal nAChRs (or DA release probability *per se)* were sufficiently higher in one region eg CPu, then following selective blockade by α-CtxMII of a subpopulation of nAChR receptors (ie α6β2*-nAChRs), cholinergic excitation at the remaining α-CtxMII-resistant nAChRs (or depolarization due to other driving factors) may be sufficient to sustain DA release probability and thus mask any apparent influence of the α6* subpopulation.

In order to eliminate these confounding explanations, we first used the AChE inhibitor, ambenonium, to enhance extracellular ACh (Zhang *et al*, 2004) to test for different (lower) ACh tone in NAc *vs* CPu. Markers of striatal cholinergic innervation (choline acetyltransferase and AChE) show dense and patchy distributions within both dorsal and ventral striatum (Graybiel *et al*, 1986; Holt *et al*, 1997; Phelps and Vaughn, 1986; Zahm and Brog, 1992) but there is not a consensus from microdialysis reports for the comparative ACh tone (eg Ichikawa *et al*, 2002; Parada *et al*, 1997). However, ambenonium in striatum has been reported to increase single-pulse-evoked DA release at low concentrations ($\sim 10^{-9}$–10^{-8} M) but suppress DA release at higher concentrations ($>10^{-8}$ M) when ACh reaches concentrations sufficient to desensitize nAChRs (Zhang *et al*, 2004). Thus, where ACh tone at nAChRs is highest, ambenonium (at higher concentrations) should more readily induce ACh-dependent nAChR desensitization. We explored DA release by a single pulse (1 p) and by a 4-p burst (100 Hz) in the presence of increasing concentrations of ambenonium (0.1 nM–10 μM). Ambenonium did not potentiate evoked $[DA]_o$ at any concentration in either CPu or NAc, and concentration-dependently suppressed $[DA]_o$ evoked by 1 p in a manner that was similar in CPu and NAc (Figure 4a). Suppression of 1 p release by ambenonium in either region was accompanied by corresponding enhancements in the ratio of $[DA]_o$ evoked by burst (4 p, 100 Hz) *vs* 1 p in a similar manner in CPu and NAc (Figure 4a). These data suggest no underlying differences in ACh tone in NAc and CPu.

Second, we compared whether other driving forces on release probability of DA differ between CPu and NAc by assessing the dependence of DA release evoked by a single pulse on stimulation current. Reductions in current (from $I = 650$ to 35 μA) gradually reduced $[DA]_o$ to zero (or below limit of detection) and in a manner that was similar in CPu and NAc (Figure 4b). These data indicate that the greater role of α6*-nAChRs in NAc is not due to differences in the net driving force on DA release probability. (Note that the absolute magnitude of effect of α6*-nAChR antagonism

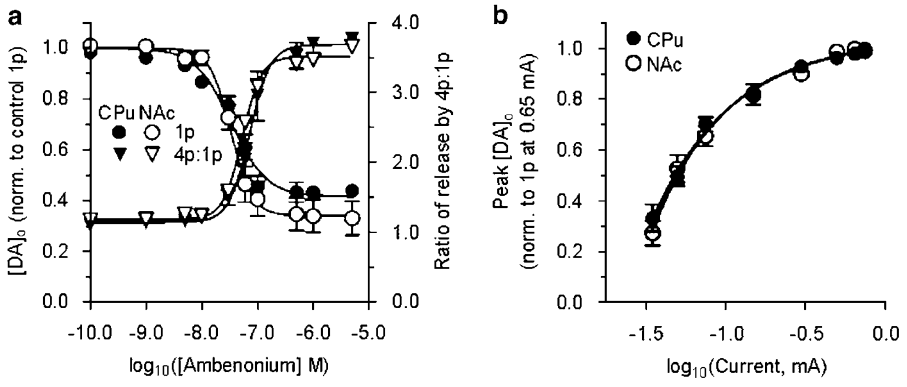

Figure 4 (**a**) No apparent variation in endogenous ACh tone and DA release probability in NAc *vs* CPu. *Circles*, Mean peak $[DA]_o \pm$ SEM evoked by 1 p as a function of concentration of the AChE inhibitor ambenonium in CPu (*filled circles*, $n = 9$) and NAc (*open circles*, $n = 9$). $[DA]_o$ are normalized to control 1 p. *Triangles*, Corresponding covariation in ratio of release by a burst (4 p, 100 Hz) *vs* a single pulse (*right-hand y-axis*) as a function of ambenonium concentration in CPu (*filled*) and NAc (*unfilled*). Curve-fits are sigmoidal ($R^2 = 0.8$–0.98, maximum constrained to 100%): For 1 p data, curve-fits and IC_{50}s are not significantly different (32 nM, CPu; 34 nM, NAc). (b) Mean peak $[DA]_o \pm$ SEM evoked by 1 p of varying stimulation current in CPu (*filled circles*, $n = 11$–98) and NAc (*open circles*, $n = 11$–100). $[DA]_o$ are normalized to 1 p release at perimaximal stimulation current, I_{max} ($I_{max} = 0.65$ mA). Curve-fits are exponential rise to maximum ($R^2 > 0.99$, maximum constrained to 100%; comparison of curves: $P < 0.05$).

does however vary dynamically in both NAc and CPu with varying depolarizing conditions, see Supplementary Results and Supplementary Figures S1, S2.)

DISCUSSION

β2*-nAChRs Operate a Filter on DA Release Probability in CPu and NAc

At concentrations seen in the plasma of tobacco smokers, nicotine via desensitization of striatal β2*-nAChRs (Zhou *et al*, 2001) enhances DA release in response to high frequency, reward-related bursts (Rice and Cragg, 2004; Zhang and Sulzer, 2004) and thus may enhance the 'salience' or processing of reward-related information. In the current study, we reveal that α6β2*-nAChRs are the receptors most responsible for these effects in the NAc, but not in the CPu. These data offer a potential cell-selective target for future therapy in nicotine addiction.

Dominant Role for α6 Subunit in NAc but not CPu

The effects of the α6*-nAChR antagonist, α-CtxMII, revealed a function for α6β2*-nAChRs in the regulation of DA release by endogenous striatal ACh in CPu and NAc, but with a striking dominance in the NAc. In CPu, the effects of α-CtxMII were a modest fraction of those seen with nicotine or the nAChR antagonist DHβE. α-CtxMII slightly diminished DA release probability by single pulses, marginally relieved short-term depression during bursts, and correspondingly, only modestly enhanced the sensitivity of DA release to presynaptic frequency. In NAc by contrast, the effects of α-CtxMII on DA release probability, and burst and frequency sensitivity were dramatic and indistinguishable from DHβE. These observations suggest that the β2*-nAChRs activated by endogenous ACh (and desensitized by nicotine) in CPu include a small population of α6β2*-nAChRs and other non-α6,β2*-nAChR populations, whereas in NAc these receptors are predominantly α6β2*-nAChRs.

The most simple explanation for the regional distinction in α6*-nAChR function revealed here could be that a greater proportion of β2*-nAChRs activated by endogenous ACh (and desensitized by nicotine) contain the α6-subunit (ie α4α6β2β3- or α6β2β3-nAChRs) in NAc than CPu. We found no evidence for competing hypotheses that the more dominant role of α6β2*-nAChRs in NAc could be otherwise explained by variations in ACh tone or in the sensitivity of DA axon membranes to depolarization. Thus, these functional differences most likely reflect a different stoichiometric composition of the nAChRs activated by endogenous ACh in striatum that regulate DA release and in turn, govern the effects of nicotine.

A function for α6β2*-nAChRs is in keeping with ligand binding and immunoprecipitation studies that indicate that α6-subunits may exist in up to 40% of β2*-nAChRs in dopaminergic axon terminals in rat CPu (Zoli *et al*, 2002) including α6β2β3 or α4α6β2β3 nAChRs (Champtiaux *et al*, 2003; Salminen *et al*, 2004; Zoli *et al*, 2002). Nicotine-evoked release of [^{3}H]-DA from rodent striatal synaptosomes and slices is also partially inhibited by α-CtxMII (Champtiaux *et al*, 2003; Grady *et al*, 2002; Kaiser *et al*, 1998; Kulak *et al*, 1997; Salminen *et al*, 2004). However, work to date has not previously been able to address the striking functional dominance of α6*-nAChRs in the control of DA release in NAc that we reveal here. For example, nAChR subunit expression data in the DA cell body regions (VTA and SNc) that give rise to ascending DA projections to NAc and CPu appears similar between cell groups (for non-α7 subunits) (Azam *et al*, 2002; Azam *et al*, 2007; Champtiaux *et al*, 2003; Klink *et al*, 2001; Wooltorton *et al*, 2003) and studies of nicotine-evoked [^{3}H]-DA release report a α-CtxMII-sensitive fraction of release that is similar in synaptosomes derived from NAc or CPu (rodents: 40–55% (Champtiaux *et al*, 2003; Grady *et al*, 2002); monkeys: 70–80% (McCallum *et al*, 2005)). However, differences between nicotine-evoked [^{3}H]-DA release studies and the physiological nAChR function identified in this study in an endogenous setting should be expected. Nicotine-evoked [^{3}H]-DA release studies probe the nAChRs available to mediate an agonist secretagogue effect of nicotine on the release of exogenous DA; by contrast, our approach has unique access to the nAChRs that are tonically activated by physiological levels of endogenous ACh, that regulate release probability of endogenous DA by action potentials and that will be desensitized by nicotine during smoking. Our data are the first to be able to reveal this dominance of α6*-nAChR function in NAc. It does not however preclude the existence of non-α6,β2*-nAChRs in DA axons in NAc. Rather, it suggests that if populations of α-CtxMII-insensitive-nAChRs exist in DA axons in NAc, they are relatively redundant compared to α6β2*-nAChRs in the functional nAChR-regulation of action potential-evoked DA release.

While these regional differences primarily identify a nAChR that offers a potential means for differentially modifying ACh–DA and nicotine–DA interactions in ventral *vs* dorsal striatum, it is possible that these data might also impact on how synchronized *pauses* in cholinergic neurons that occur in response to reward-related cues (Morris *et al*, 2004) govern DA function (Cragg, 2006). Differences (albeit currently unresolved) in affinity for ACh of each receptor type might have some impact on the efficacy or speed (and therefore time window) with which a fall and rise in striatal ACh tone at nAChRs would be detected and impact on striatal DA release following a synchronized pause in ACh neurons.

SUMMARY AND CONCLUSIONS

Striatal β2*-nAChRs play a key role in the powerful effects of nicotine in governing how DA neuron activity is relayed in to DA release. Thus nicotine, via nAChR desensitization, can promote DA signaling by high frequency, reward-related activity (Rice and Cragg, 2004; Zhang and Sulzer, 2004) and thus participate in the signaling and learning of reward-related information which ultimately participates in nicotine dependence. The current data now indicate that α6*-nAChRs play a key component function in this dynamic frequency filtering of DA release probability by endogenous ACh and nicotine. Furthermore, these data reveal for the first time that α6*-nAChRs dominate in the effects of nicotine in the NAc. As a consequence, targeting of α6β2* or non-α6β2* nAChRs could differentially modulate the

components of behavioral reinforcement and sensorimotor function with which ventral and dorsal striatum are differently associated. The discrete localization within the brain of α6*-nAChRs to catecholaminergic neurons, in conjunction with these key distinctions in α6*-nAChR control of DA neurotransmission between different DA systems, suggests that α6*-nAChRs may offer a powerful molecular target for a highly selective future pharmacotherapeutic or genetic strategy to combat nicotine addiction.

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