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Long-Term Nicotine Treatment Differentially Regulates Striatal $\alpha6\alpha4\beta2^*$ and $\alpha6(\text{Non}\alpha4)\beta2^*$ nAChR Expression and Function

Xiomara A. Perez, Tanuja Bordia, J. Michael McIntosh, Sharon R. Grady, and Maryka Quik

The Parkinson's Institute, Sunnyvale, California (X.A.P., T.B., M.Q.); Department of Biology and Psychiatry, University of Utah, Salt Lake City, Utah (J.M.M.); and Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado (S.R.G.).

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ABSTRACT

Nicotine treatment has long been associated with alterations in $\alpha4\beta2^*$ nicotinic acetylcholine receptor (nAChR) expression that modify dopaminergic function. However, the influence of long-term nicotine treatment on the $\alpha6\beta2^*$ nAChR, a subtype specifically localized on dopaminergic neurons, is less clear. Here we used voltammetry, as well as receptor binding studies, to identify the effects of nicotine on striatal $\alpha6\beta2^*$ nAChR function and expression. Long-term nicotine treatment via drinking water enhanced nonburst and burst endogenous dopamine release from rat striatal slices. In control animals, $\alpha6\beta2^*$ nAChR blockade with α-conotoxin MII (α-CtxMII) decreased release with nonburst stimulation but not with burst firing. These data in control animals suggest that varying stimulus frequencies differentially regulate $\alpha6\beta2^*$ nAChR-evoked dopamine release. In contrast, in nicotine-treated rats, $\alpha6\beta2^*$ nAChR blockade elicited a similar pattern of dopamine release with nonburst and burst firing. To elucidate the $\alpha6\beta2^*$ nAChR subtypes altered with long-term nicotine treatment, we used the novel α-CtxMII analog E11A in combination with $\alpha4$ nAChR knockout mice. ^{125}I-α-CtxMII competition studies in striatum of knockout mice showed that nicotine treatment decreased the $\alpha6\alpha4\beta2^*$ subtype but increased the $\alpha6(\text{non}\alpha4)\beta2^*$ nAChR population. These data indicate that $\alpha6\beta2^*$ nAChR-evoked dopamine release in nicotine-treated rats is mediated by the $\alpha6(\text{non}\alpha4)\beta2^*$ nAChR subtype and suggest that the $\alpha6\alpha4\beta2^*$ nAChR and/or $\alpha4\beta2^*$ nAChR contribute to the differential effect of higher frequency stimulation on dopamine release under control conditions. Thus, $\alpha6\beta2^*$ nAChR subtypes may represent important targets for smoking cessation therapies and neurological disorders involving these receptors such as Parkinson's disease.

Nicotine, the primary reinforcing component in tobacco, exerts its effects in large part by acting on dopamine neurons (Wonnacott et al., 2005; Dani and Bertrand, 2007). This includes the mesolimbic and nigrostriatal dopaminergic systems that originate in the ventral tegmental area and substantia nigra and project to the nucleus accumbens and striatum, respectively. Dopaminergic nerve terminals synapse on multiple targets in these regions including cholinergic interneurons. These neurons are tonically active and continually release acetylcholine, which subsequently affects dopaminergic function (Zhou et al., 2002). The mesolimbic dopamine system is primarily involved in reward and reinforcement (Wonnacott et al., 2005; Dani and Bertrand, 2007), whereas nigrostriatal dopamine neurons are involved in addictive behaviors and the control of movement (Janhunen and Ahtee, 2007; Singh et al., 2007).

Nicotine exerts its effects on dopaminergic function by interacting with nicotinic acetylcholine receptors (nAChRs), of which there are multiple subtypes (Wonnacott et al., 2005; Dani and Bertrand, 2007). One of these is the $\alpha4\beta2^*$ nAChR population, which consists of $\alpha4\beta2$ and $\alpha4\alpha5\beta2$ subtypes, as well as other minor subtypes (the asterisk indicates the possible presence of other nicotinic subunits in the receptor complex) (Gotti et al., 2007; Grady et al., 2007; Quik et al., 2007). It is noteworthy that long-term nicotine treatment differentially influences $\alpha4\beta2^*$ nAChR subtype expression, with an increase in $\alpha4\beta2$ sites and no change in $\alpha4\alpha5\beta2$ nAChRs (Mao et al., 2008). These alterations in $\alpha4\beta2^*$ nAChR expression most likely influence reward-related behaviors (Tapper et al., 2004; Maskos et al., 2005) and the locomotor effects of nicotine (Marubio et al., 2003; Tapper et al., 2007).

The involvement of $\alpha6\beta2^*$ nAChRs in behavior is less well studied than that for $\alpha4\beta2^*$ nAChRs; however, accumulating evidence suggests this subtype also plays an important role.

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ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; α-CtxMII, α-conotoxin MII; ANOVA, analysis of variance.

Several major α6β2* nAChRs have been identified in striatum, including the α6α4β2* and α6(nonα4)β2* subtypes (Salminen et al., 2004; Gotti et al., 2007; Grady et al., 2007; Quik et al., 2007). These receptors are selectively localized to catecholaminergic regions and represent 20 to 45% of nAChRs in the mesolimbic and nigrostriatal systems, depending on species (Quik and McIntosh, 2006). In addition, [^{3}H]dopamine release studies show that α6β2* nAChRs mediate 30 to 75% of the response in striatum and nucleus accumbens (Quik and McIntosh, 2006; Grady et al., 2007), whereas voltammetry studies indicate that α6β2* nAChRs are responsible for the greater majority of dopamine release in the nucleus accumbens (Exley et al., 2008). These data suggest that α6β2* nAChRs may also mediate effects of nicotine on behaviors linked to dopamine function, including addiction and motor control. Indeed, it has been shown that α6β2* nAChRs are regulated by nicotine treatment, but in a manner distinct from α4β2* nAChRs. Lont-term nicotine treatment leads to a down-regulation or no change in α6β2* nAChR expression, although studies to determine selective effects on the α6α4β2* and α6(nonα4)β2* subtypes remain to be evaluated.

Because nicotine exposure in humans via smoking or drug treatment is generally long term, we investigated changes in striatal endogenous dopamine release and α6β2* nAChR subtype expression after long-term nicotine administration in rodents. Our results show that nicotine treatment results in an up-regulation of the α6(nonα4)β2* subtype but a loss of α6α4β2* nAChRs, suggesting that the α6(nonα4)β2* subtype primarily modulates α6β2* nAChR-mediated dopamine release after long-term nicotine administration. These results have implications for the development of subtype selective nicotinic agonists for smoking cessation and the treatment of neurological disorders.

Materials and Methods

Mouse Studies. Mice engineered to contain a null mutation in the α4 nAChR subunit gene, obtained from the laboratory of Dr. John Drago (Ross et al., 2000), were maintained and bred at the Institute for Behavioral Genetics, University of Colorado (Boulder, CO). A 12-h light/dark cycle was used with room temperature at 22°C, during which the mice had free access to food and water. Mice weaned at 25 days of age were housed with same-sex littermates. The genotype was determined at 40 days of age by polymerase chain reaction (Salminen et al., 2004) using isolated tail DNA. Mice used for the present study were age-matched littermates of mixed genetic background: wild type and α4(−/−). Nicotine was added to the saccharin-containing solution starting at an initial concentration of 25 μg/ml. This dose was gradually increased to a final dose of 300 μg/ml over a period of 10 days. The animals were maintained on the final dose of nicotine for 14 days, and then killed by cervical dislocation. All procedures were in accordance with guidelines and approval of the Animal Care and Use Committee of the University of Colorado, Boulder.

The brains were removed and immediately frozen in isopentane on dry ice and stored at −80°C. Sections (8 μm) were prepared using a cryostat (Leica Microsystems, Inc., Deerfield, IL) cooled to −20°C. Frozen sections were thaw-mounted onto Superfrost Plus slides (Fisher, Pittsburgh, PA), air-dried, and stored at −80°C for nicotinic receptor autoradiography.

Rat Studies. Adult male Sprague-Dawley rats (250–300 g) were purchased from Charles River Laboratories (Gilroy, CA). Rats were placed in a temperature-controlled room with a 12-h dark/light cycle and were housed 2 to 3 per cage. All animals had free access to food and water. They were randomly divided into different treatment groups 2 days after arrival. After several days of acclimatization, rats were given drinking water containing 2% saccharin in the initial studies and 1% in subsequent experiments (Sigma Chemical Co., St. Louis, MO) to mask the bitter taste of nicotine. Nicotine was added to the saccharin-containing solution of the treated group, with the nicotine saccharin solution adjusted to pH 7.0 throughout the course of the study. Rats were started at an initial concentration of 25 μg/ml nicotine (free base; Sigma, St. Louis, MO) in the drinking water, which was increased to 50 μg/ml on days 5 and 6, and to a final dose of 100 μg/ml on days 8 to 10. The animals were maintained at this final dose for 14 days. Fluid intake and weights were monitored daily, and there were no significant differences between the two treatment groups. Nicotine was removed from the drinking water 18 h before death to minimize nicotine tissue levels before the functional assays. The rats were killed by decapitation using a guillotine. All procedures conform to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and were approved by the Animal Care and Use Committee of the Parkinson's Institute.

The brain was quickly removed and chilled in ice-cold, preoxygenated (95% O_2/5% CO_2) physiological buffer containing 125 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 2.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, 20 mM HEPES, 11 mM glucose, and 25 mM $NaHCO_3$, pH 7.4. Corticostriatal slices (400 μm thick) were cut using a vibrating blade microtome (VT1000S; Leica Microsystems, Bannockburn, IL) and incubated at room temperature in oxygenated buffer. The remaining portion of the brain, which contained the mid to posterior striatum, was quick frozen in isopentane on dry ice immediately after the sections were removed, and stored at −80°C. Sections (8 μm) were prepared using a cryostat (Leica Microsystems) cooled to −20°C. Frozen sections were thaw-mounted onto Superfrost Plus slides (Thermo Fisher Scientific, Waltham, MA), air-dried, and stored at −80°C for nicotinic receptor autoradiography.

Electrochemical Measurement of Dopamine Release. For the fast-scan cyclic voltammetry experiments, carbon fiber microelectrodes were constructed as described previously (Perez et al., 2008). The electrode potential was linearly scanned from 0 to −400 to 1000 to −400 to 0 mV versus an Ag/AgCl reference electrode at a scan rate of 300 mV/ms (Zhou et al., 2001; Perez et al., 2008). This triangular wave was repeated every 100 ms at a sampling frequency of 50 Hz. Current was recorded with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). Triangular wave generation and data acquisition were controlled by pClamp 9.0 software (Molecular Devices). Electrical stimulation was applied using a bipolar tungsten stimulating electrode (Plastics One, Roanoke, VA) connected to a linear stimulus isolator (WPI, Saratoga, Fl) and triggered by a Master-8 pulse generator (A.M.P.I., Jerusalem, Israel). All electrode placements were made in the dorsal striatum with the aid of a stereomicroscope and micromanipulators. Background current was digitally subtracted, and the peak oxidation currents were converted into concentration after postexperimental calibration of the carbon fiber electrode with a fresh solution of 1 μM dopamine in experimental buffer.

After at least 2 h of incubation and 30 min before recording, a brain slice was transferred to a submersion recording chamber (Campden Instruments Ltd., Lafayette, IN), perfused at 1 ml/min with 30°C, oxygenated artificial cerebrospinal fluid, and allowed to equilibrate. Dopamine release from dorsal striatum was evoked by either a single, rectangular electrical pulse (4 ms) applied every 2.5 min or by a burst of four pulses at 30 Hz applied every 5 min with a stimulus intensity of 8 V that achieved 60% maximal release. The recording sites were always restricted to the same area of the dorsal striatum to ensure consistency of the signals across animals. Evoked release by both a single pulse and a burst of pulses was first assessed in physiological buffer. α-CtxMII (100 nM) prepared in physiological buffer was then perfused through the slice; release evoked by a single

pulse and then through a burst of pulses were continuously monitored. This concentration of α-CtxMII maximally blocks α6β2* nAChRs without affecting other nAChR subtypes (Exley et al., 2008).

^{125}I-Epibatidine Autoradiography. Binding of ^{125}I-epibatidine (2200 Ci/mmol; PerkinElmer Life and Analytical Sciences, Waltham, MA) was performed as reported previously (Kulak et al., 2002). Slides were preincubated at 22°C for 15 min in buffer containing 50 mM Tris, pH 7.5, 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, and 1.0 mM $MgCl_2$. They were incubated for 40 min with 0.015 nM ^{125}I-epibatidine in the presence or absence of α-CtxMII (300 nM). They were then washed, dried, and exposed to Kodak MR film with ^{125}I standards for several days. Nonspecific binding was assessed in the presence of 100 μM nicotine and was similar to the film blank.

^{125}I-α-CtxMII Autoradiography. ^{125}I-α-CtxMII (from J. M. McIntosh) was synthesized and radiolabeled as described previously (Whiteaker et al., 2000). Binding of ^{125}I-α-CtxMII (specific activity, 2200 Ci/mmol) was done as reported previously (Quik et al., 2001). Thawed 8-μm striatal sections were preincubated at room temperature for 15 min in binding buffer (144 mM NaCl, 1.5 mM KCl, 2 mM $CaCl_2$ 1 mM $MgSO_4$, 20 mM HEPES, and 0.1% bovine serum albumin, pH 7.5) plus 1 mM phenylmethylsulfonyl fluoride along with different concentrations (1.0 fM–0.1 μM) of α-CtxMII analog E11A. This was followed by 1-h incubation at room temperature in binding buffer also containing 0.5% bovine serum albumin, 5 mM EDTA, 5 mM EGTA, and 10 μg/ml each aprotinin, leupeptin, and pepstatin A plus 0.5 nM ^{125}I-α-CtxMII, as well as the competing concentrations of E11A (Bordia et al., 2007). The assay was terminated by washing the slides for 10 min at room temperature, 10 min in ice-cold binding buffer, twice for 10 min in 0.1× buffer at 0°C, and two final 5-s washes in ice-cold deionized water. The striatal sections were air-dried and exposed to Kodak BioMax MR film (Carestream Health, Rochester, NY) for 2 to 5 days together with ^{125}I-microscale standards (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK). Nicotine (100 μM) was used to determine nonspecific binding.

Data Analyses. To evaluate optical density values from autoradiographic films, we used the ImageQuant program from GE Healthcare. To assess specific binding of the radioligands, background tissue levels were first subtracted from total binding to the tissue. The resultant values were converted to femtomole per milligram of tissue using standard curves determined from ^{125}I standards. The optical density readings of the samples were always within the linear range of the film. For analyses of striatal values, the ventral striatum (i.e., nucleus accumbens) was always excluded, as there may be differences in the nAChR population between dorsal and ventral striatum.

All curve fittings and statistics were conducted using Prism software (Graph Pad Software Co., San Diego, CA). Statistical comparisons were performed using either Student's *t* test or two-way analysis of variance (ANOVA) followed by Bonferroni post hoc test (Prism) because this test is conservative and allows for comparison of all pairs of data. A value of $p < 0.05$ was considered significant. All values are expressed as the mean ± S.E.M. of the indicated number of animals.

Results

Effect of Long-Term Nicotine Treatment via the Drinking Water on nAChR Expression in Rat Striatum. For the present experiments, we chose a long-term nicotine treatment paradigm via the drinking water, because it allows for prolonged, intermittent exposure to nicotine (Pietilä and Ahtee, 2000; Lai et al., 2005). Experiments were first done to determine the effects of long-term nicotine treatment on overall receptor expression (Fig. 1). Quantitative receptor autoradiography using ^{125}I-epibatidine, a ligand that labels multiple nAChR subtypes, was done in the presence of α-CtxMII to identify α-CtxMII-resistant ^{125}I-epibatidine (α4β2* nAChRs) and α-CtxMII-sensitive ^{125}I-epibatidine binding sites (α6β2* nAChRs). Oral nicotine treatment significantly increased α4β2* ($p < 0.05$) and decreased α6β2* ($p < 0.05$) nAChRs in the striatum of rats (Fig. 1, A and B) as previously reported in mice and monkeys (Lai et al., 2005; McCallum et al., 2006). To directly measure α6β2* nAChR, we performed ^{125}I-α-CtxMII binding studies. Nicotine treatment also significantly decreased ^{125}I-α-CtxMII binding ($p < 0.001$), indicating that ^{125}I-α-CtxMII labeled sites identify the same subset of α6β2* nAChRs as α-CtxMII-sensitive ^{125}I-epibatidine binding sites (Fig. 1C).

Long-Term Nicotine Treatment via the Drinking Water Increases Electrically Evoked Dopamine Release in Rat Dorsal Striatum. In the present studies, we used voltammetry to evaluate the effect of long-term nicotine

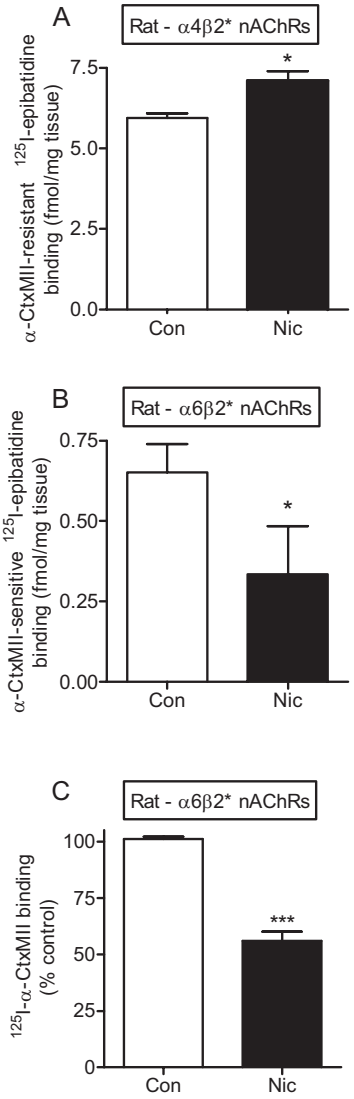

Fig. 1. Long-term nicotine treatment differentially regulates α4β2* and α6β2* nAChRs in rat striatum. Rats were given nicotine in the drinking water for 14 days, after which receptor autoradiography was done using ^{125}I-epibatidine and ^{125}I-α-CtxMII. Changes in the α4β2* and α6β2* nAChRs were determined by measurement of ^{125}I-epibatidine binding in the absence and presence of α-CtxMII (300 nM), with an increase in α4β2* nAChRs or α-CtxMII-resistant ^{125}I-epibatidine binding sites (A) and a decrease in α6β2* nAChRs or α-CtxMII-sensitive ^{125}I-epibatidine binding sites (B). α6β2* nAChRs were also measured using ^{125}I-α-CtxMII, resulting in a significant decline with nicotine treatment (C). Data represent mean ± S.E.M. of six to eight rats. Significance of difference from control using a *t* test, *, $p < 0.05$; ***, $p < 0.001$.

treatment on nAChR-mediated dopaminergic function for several reasons. One of these was that it provides a measure of dopamine release from a slice preparation, in which the modulatory influence of other neurotransmitter systems is still partially intact. In addition, voltammetry involves release of endogenous acetylcholine to stimulate nAChRs and subsequently assesses responses in endogenous dopamine release. Voltammetry also offers an excellent temporal resolution (milliseconds) and allows for a determination of dopaminergic activity at varying firing frequency. This is important because nicotine's effect on dopamine release is dependent on the frequency of stimulation of dopaminergic neurons (that is, tonic versus phasic stimulation) (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Exley et al., 2008; Meyer et al., 2008).

Electrochemical studies to study alterations in dopamine neurotransmission after nicotine exposure have thus far only investigated dopamine release after short-term tissue application (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004; Exley et al., 2008). To evaluate the effects of long-term nicotine treatment on endogenous dopamine release from striatal slices, we first examined the effects of a single pulse (nonburst firing) versus a four-pulse (burst firing) stimulus on dopamine release in slices from control animals. Single pulse stimulation elicited a rapid release of dopamine as characterized by its typical voltammogram showing an oxidation peak at approximately 500 to 600 mV and a reduction peak at approximately −200 mV (Fig. 2, inset). An increase in the number of stimulus pulses (four pulses at 30 Hz) yielded a similar level of dopamine release compared with a single-pulse stimulus (0.17 ± 0.01 and 0.19 ± 0.01 μM for one- and four-pulse stimulation, respectively) (Fig. 2). These results support the idea that short-term depression in response to burst stimuli decreases the amount of dopamine release with each successive pulse, thus tapering total release after a burst of pulses in both control and nicotine-treated rats (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004; Exley et al., 2008)

Long-term nicotine treatment significantly enhanced endogenous dopamine release (Fig. 2) with both one- and four-pulse stimulation as assessed using two-way ANOVA by stimulus pattern and treatment. The increase in dopamine release was similar (~50%) for the nicotine-treated rats compared with control with one-pulse (from 0.17 ± 0.01 to 0.25 ± 0.02 μM) and four-pulse (from 0.19 ± 0.01 to 0.26 ± 0.02 μM) stimulation. These findings indicate that burst-stimulated dopamine release is still affected by short-term depression after long-term nicotine treatment.

Long-Term Nicotine Treatment Prevents the Enhancement of Dopamine Release with Burst Firing. To determine the involvement of the α6β2* nAChR subtype in the release of endogenous dopamine from striatal slices in control rats, we used the specific α6β2* nAChR antagonist α-CtxMII. Release was elicited by either a single pulse or a four-pulse stimulus to probe the relationship between firing pattern and α6β2* nAChR-mediated dopamine function. The identity of the released neurotransmitter was confirmed by the characteristic voltammograms (i.e., an oxidation peak at approximately 500 to 600 mV and a reduction peak around −200 mV; see Fig. 3, insets). Addition of α-CtxMII to the perfusion media resulted in a significant decrease ($p < 0.001$) in dopamine release with nonburst stimuli (Fig. 3), whereas α-CtxMII significantly increased ($p < 0.05$) peak dopamine release under burst-firing conditions, as analyzed using two-way ANOVA by stimulus pattern and α-CtxMII exposure (Fig. 3). These results are in agreement with a recent report showing that blockade of α6β2* nAChRs significantly increases the sensitivity of dopamine release to burst versus nonburst stimuli (Exley et al., 2008).

We next used α-CtxMII to assess the effect of long-term nicotine treatment on α6β2* nAChR-mediated dopamine release. In nicotine-treated animals, addition of α-CtxMII to the perfusion buffer led to a significant 48% decrease ($p < 0.001$) in endogenous dopamine release evoked by a single pulse (Fig. 3), similar to results in control rats, in which a

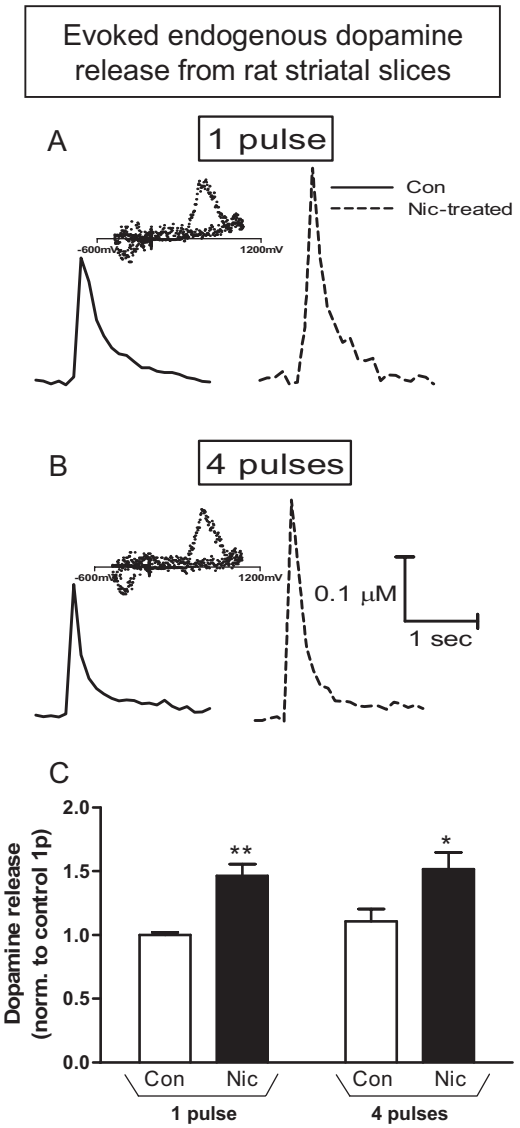

Fig. 2. Long-term nicotine treatment increases electrically evoked dopamine release in rat striatum. Top and middle, representative traces of dopamine release in striatum of control (solid line) and nicotine-treated (dashed line) rats after one- and four-pulse stimulation, respectively. Insets, typical voltammograms for dopamine with an oxidation peak at 500 to 600 mV and a reduction peak at approximately −200 mV. Quantitative analyses of peak dopamine release (bottom) for each treatment group normalized to release by one pulse in control animals. The values represent the mean ± S.E.M. of four to six rats (15–25 observations per animal). Significance of difference from control using a Bonferroni post hoc test after a one-pulse stimulus, **, $p < 0.01$; after a four-pulse stimulus, *, $p < 0.05$.

53% decline in dopamine release was observed, as analyzed using two-way ANOVA by stimulus pattern and α-CtxMII exposure. These data suggest that there are functional changes in other nAChR subtypes, such as the α4β2* nAChR, with long-term nicotine dosing. In contrast to the effect of α6β2* nAChR blockade on burst-evoked dopamine release in the control rats (Fig. 3), there was a significant 61% decrease ($p < 0.001$) in dopamine release with burst stimulation in nicotine-treated animals (Fig. 3). These results suggest that long-term nicotine treatment modifies the α6β2* nAChR-mediated regulation of striatal dopaminergic function.

Further analyses were then done to assess the effects of long-term nicotine compared with the saccharin control with single-pulse and burst stimulation in the presence of α-CtxMII. Two-way ANOVA showed that there was a significant main effect of nicotine treatment ($p < 0.05$) and stimulus pattern ($p < 0.001$). In addition, there was a significant interaction ($p < 0.001$), with an increase in dopamine release with the one pulse stimulus but a decrease in release with the four-pulse stimulus. These results further highlight the prominent changes that occur in nicotinic subtype-evoked dopamine release with nicotine treatment.

Preferential Down-Regulation of the α6α4β2* or Very-High-Affinity α6β2* nAChR Subtype in Striatum of Rats Receiving Long-Term Treatment with Nicotine. Our previous study using the α-CtxMII analog E11A showed that there are two major α6β2* nAChR subtypes in mouse striatum, the α6α4β2* and the α6(nonα4)β2* nAChRs in agreement with previous findings (Salminen et al., 2004; Bordia et al., 2007). To investigate whether E11A could also distinguish these subtypes in rat striatum, ^{125}I-α-CtxMII competition assays were done with varying concentrations of E11A using serial striatal sections from control rats. E11A inhibition of ^{125}I-α-CtxMII binding yielded a two-site inhibition curve (data best fit to a two-site model) with a ~1800-fold difference in affinity between sites (Fig. 4, Table 1). The very-high-affinity fraction represented 51% of the total E11A sensitive ^{125}I-α-CtxMII binding sites (Table 1). In rats undergoing long-term nicotine treatment, competition analysis of E11A inhibition of ^{125}I-α-CtxMII binding yielded a monophasic curve in four animals and a biphasic curve in two animals. This diversity between animals could relate to individual variations in outbred rats that influence the effect of nicotine treatment (Fagen et al., 2007). When all the data were pooled, a biphasic curve was obtained showing a preferential decrease in the very-high-affinity f1 fraction (control, f1 = 74%; nicotine, f1 = 26%) (Fig. 4, Table 1).

Long-Term Nicotine Treatment Differentially Regulates α6α4β2* and α6(Nonα4)β2* Subtypes in Mouse Striatum. The experiments described in the preceding sections were done with rats as one of our long-term objectives is to evaluate effects of nicotine on behavioral measures, which

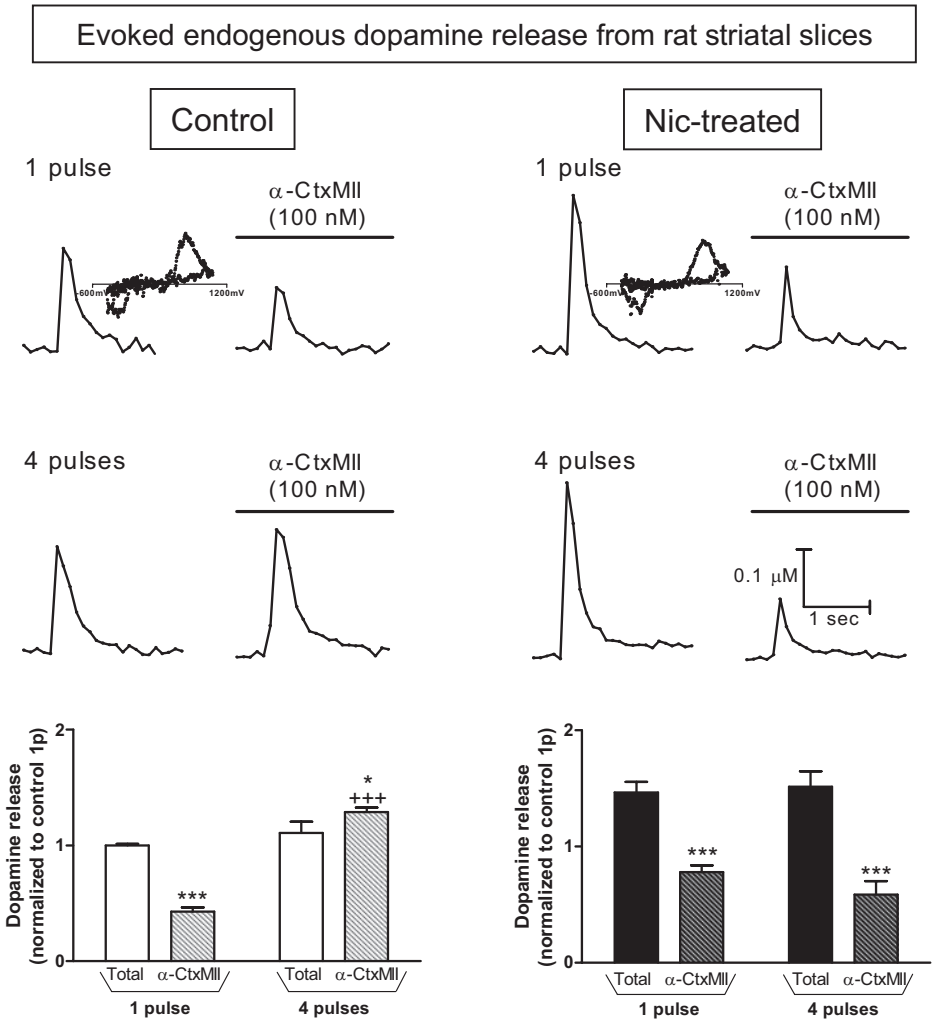

Fig. 3. Long-term nicotine treatment prevents the enhancement of burst-stimulated dopamine release after α6β2* nAChR blockade. Representative traces for dopamine release after one-pulse (top) and four-pulse (middle) stimulation in the absence and presence of α-CtxMII for both control (left) and nicotine-treated (right) rats. Insets, typical voltammograms for dopamine with oxidation peaks at 500 to 600 mV and reduction peaks at approximately −200 mV. Quantitative analyses (bottom) of peak dopamine release normalized to control total release by one pulse in control (left) and nicotine-treated (right) rats induced by nonburst and burst stimulation before and after application of α-CtxMII. The values represent the mean ± S.E.M. of six rats (15–20 observations per animal). Significance of difference from total release using a Bonferroni post hoc test, *, $p < 0.05$; ***, $p < 0.001$. Significance of difference from release with one-pulse stimulation in the presence of α-CtxMII using a Bonferroni post hoc test, $^{+++}$, $p < 0.001$.

may more readily be performed in this species. However, because the use of knockout mice often provides clearer insight about the specific α6β2* nicotinic receptor subtypes (α6α4β2* or/and α6(nonα4)β2*) involved in functional effects of nicotine, we next initiated a series of studies in wild-type and α4 nAChR-null mutant mice. Such an approach seemed reasonable because the magnitude of α6β2* nAChR expression and function is similar in rat and mouse striatum. The α6β2* nAChR subtype represents approximately 15 to 20% of the nAChR population in both rat or mouse striatum and is responsible for ~30% of evoked [^{3}H]dopamine release from striatal synaptosomes in both rat and mouse (Quik and McIntosh, 2006).

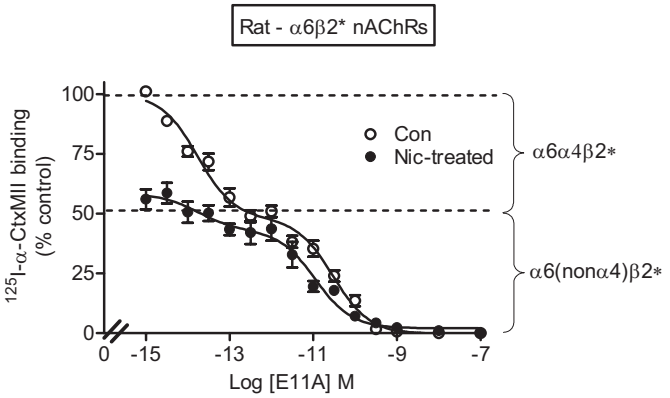

Fig. 4. Long-term nicotine treatment preferentially down-regulates a subpopulation of α6β2* nAChRs in rat striatum. To further characterize the effect of nicotine treatment on α6β2* nAChRs, ^{125}I-α-CtxMII competition assays were done using a range of concentrations (10^{-15}–10^{-7} M) of the α-CtxMII analog E11A. A biphasic inhibition curve (data fit best to two site model) was obtained in control striatum indicating that E11A discriminates between at least two α6β2* nAChRs, previously shown to represent the α6α4β2* and α6(nonα4)β2* subtypes. Competition analysis of E11A inhibition of ^{125}I-α-conotoxin MII binding in striatum of rats receiving nicotine showed a preferential decline in the very-high-affinity α6β2* nAChR(that is, the α6α4β2* subtype). Symbols represent mean ± S.E.M. of six to eight rats. Where the S.E.M. is not depicted, it fell within the symbol.

^{125}I-Epibatidine binding in the absence and presence of α-CtxMII was used to distinguish between α4β2* and α6β2* binding sites. Results in Fig. 5, analyzed using two-way ANOVA by genotype and nicotine treatment, show a significant increase ($p < 0.001$) in α4β2* nAChRs in wild-type mice with nicotine treatment (Fig. 5A). In contrast, no binding to α4β2* nAChRs was observed in striatum of α4(−/−) mice, as expected because the α4 subunit is not expressed in α4(−/−) mice. α6β2* nAChR sites were significantly decreased ($p < 0.01$) with nicotine treatment in wild-type mice. We were surprised to find that there was a significant increase in the α-CtxMII-sensitive ^{125}I-epibatidine binding component in the striatum of α4(−/−) mice receiving nicotine. Because α4(−/−) mice do not express α6α4β2* nAChRs, these data suggest there is a selective up-regulation of α6(nonα4)β2* nAChRs with nicotine treatment.

^{125}I-α-CtxMII binding was used as an alternate measure to study changes in striatal α6β2* nAChRs with nicotine treatment in wild-type and α4(−/−) mice (Fig. 5C). Long-term treatment with oral nicotine decreased α6β2* ($p < 0.01$) nAChRs in wild-type mice. ^{125}I-α-CtxMII binding was also decreased in α4(−/−) mice, indicating a loss in α6α4β2* subtype as predicted in α4(−/−) mice. Again, with long-term nicotine treatment there was a significant increase ($p < 0.01$) in α6β2* binding in α4(−/−) mice. These data again confirm the up-regulation of α6(nonα4)β2*nAChRs with nicotine treatment.

To directly determine the effects of nicotine on the α6α4β2* and α6(nonα4)β2* nAChR subtypes in mouse striatum, we performed ^{125}I-α-CtxMII competition assays using a range of concentrations (10^{-15}–10^{-7} M) of E11A. Competition binding studies in wild-type mice yielded a two-site binding curve (Fig. 6), with IC_{50} values of 0.011 and 6.5 pM (Table 1). In the striatum of α4(−/−) mice, E11A inhibited ^{125}I-α-CtxMII binding in a monophasic manner, as expected because α4(−/−) mice do not express α6α4β2* nAChRs. It is noteworthy that ^{125}I-α-CtxMII competition curves with E11A were monophasic in wild-type and α4(−/−) mice that received

TABLE 1

Long-term nicotine treatment modulates α6α4β2* and α6(nonα4)β2* nAChR expression

^{125}I-α-CtxMII competition studies were done using striatal sections from control and nicotine-treated rats or wild-type and α4 nAChR-null mutant mice at different concentrations of E11A. Biphasic inhibition by E11A (data best fit to a two-site model) was obtained in the striatum of both control rats and wild-type, mice suggesting the presence of at least two α6β2* nAChRs. In rats receiving nicotine, competition analysis of the data also yielded a biphasic curve; however, there was a preferential decrease in the f1 fraction (f1:f2 = 26:74). In contrast, E11A competition analyses using striatum of non–nicotine-treated α4 nAChR-null mutant mice yielded monophasic curves, suggesting a loss in the very-high-affinity binding site. Similar monophasic curves were obtained in mice (wild type and α4 nAChR-null mutants) receiving nicotine. Each value represents the mean ± S.E.M. of six to eight rats or three mice. The numbers in parentheses are the 95% confidence intervals (CI).

Species & Genotype	Treatment	Preferred Model (No. of Sites)	E11A		Ratio ($IC_{50}2/IC_{50}1$)	Fraction of Receptors	
			$IC_{50}1$ (CI)	$IC_{50}2$ (CI)		f1	f2
			pM			%	
Rat							
N.A.	Control	Two	0.017 (0.014–0.021)	30 (25–35)	1765	51	49
	Nicotine	Two	0.017 (0.009–0.034)	11 (9.2–13.6)	647	26	74
Mouse							
Wild type	Control	Two	0.011 (0.002–0.032)	6.5 (4.3–9.7)	590	34	66
	Nicotine	One		2.3 (1.5–2.9)			100
α4(−/−)	Control	One		2.1 (1.3–3.9)			100
	Nicotine	One		1.1 (0.7–1.5)			100

N.A., not available.

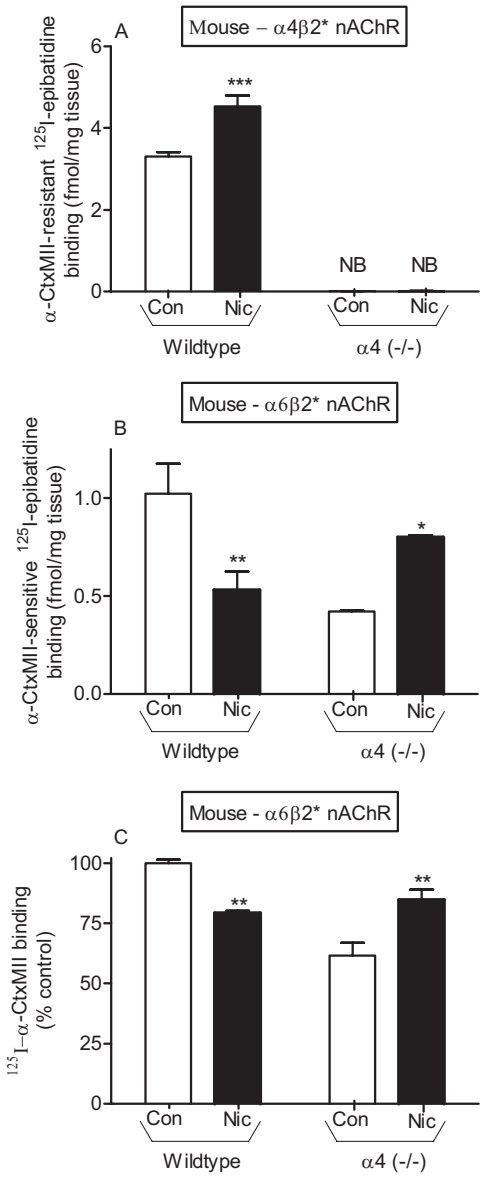

Fig. 5. Nicotine treatment differentially alters $\alpha6\beta2^*$ nAChRs in wild-type and $\alpha4(-/-)$ nAChR mice. Mice were maintained at the final dose of nicotine in the drinking water for 14 days, after which ^{125}I-epibatidine binding was done in the absence and presence of 300 nM unlabeled α-CtxMII to identify α-CtxMII-resistant ($\alpha4\beta2^*$) and α-CtxMII-sensitive ($\alpha6\beta2^*$) binding sites. A, striatal $\alpha4\beta2^*$ nAChRs in wild-type mice were significantly increased after nicotine treatment, with no binding in striatum of $\alpha4(-/-)$ mice, as expected. B, $\alpha6\beta2^*$ nAChR binding was significantly decreased in $\alpha4(-/-)$ mice and nicotine-treated wild-type mice. Unexpectedly, there was a significant increase in $\alpha6\beta2^*$ nAChR binding in striatum of $\alpha4(-/-)$ mice receiving nicotine. Because $\alpha4(-/-)$ mice do not express $\alpha6\alpha4\beta2^*$ nAChRs, these data suggest that there is a selective up-regulation of $\alpha6(non\alpha4)\beta2^*$ nAChRs with nicotine treatment. C, for a direct measure of changes in $\alpha6\beta2^*$ nAChRs with nicotine treatment, ^{125}I-α-CtxMII binding was done using striatal sections from wild-type and $\alpha4$ nAChR-null mutant mice. Again, nicotine treatment decreased $\alpha6\beta2^*$ nAChRs in the wild-type mice. There was also a decrease in the $\alpha4(-/-)$ mice due to a loss of the $\alpha6\alpha4\beta2^*$ subtype. Again, however, there was a significant increase in $\alpha6\beta2^*$ binding in nicotine-treated $\alpha4(-/-)$ mice, further supporting the idea that there is a selective up-regulation of $\alpha6(non\alpha4)\beta2^*$ nAChRs with nicotine treatment. Data represent mean ± S.E.M. of three mice. Significance of difference from control using a Bonferroni post hoc test, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. NB, no binding.

long-term treatment with nicotine. Moreover, the IC_{50} values were similar to those of the lower affinity binding site (2.3 and 1.1 pM for wild-type and $\alpha4(-/-)$ mice, respectively) (Table 1). These data suggest a preferential down-regulation of $\alpha6\alpha4\beta2^*$ and up-regulation of the $\alpha6(non\alpha4)\beta2^*$ nAChR in the striatum of mice receiving nicotine.

Discussion

The present results are the first to use cyclic voltammetry to investigate the effects of long-term nicotine treatment on the function of $\alpha6\beta2^*$ nAChRs, a subtype that evokes a large fraction of striatal nAChR-mediated dopamine release (Quik and McIntosh, 2006; Exley et al., 2008; Meyer et al., 2008). Our results in control animals show that blockade of $\alpha6\beta2^*$ nAChRs leads to a decrease in dopamine release with single pulse stimulation but an increase in release with burst stimulation in agreement with previous findings (Exley et al., 2008). It is noteworthy that long-term nicotine treatment prevented this enhanced evoked dopamine release with burst firing. Subsequent receptor binding studies to understand the nAChRs that contribute to this effect showed that long-term nicotine treatment led to a small increase in $\alpha6(non\alpha4)\beta2^*$ but resulted in a complete loss of $\alpha6\alpha4\beta2^*$ nAChRs. These novel data, coupled with the results of the functional studies, suggest that $\alpha6\alpha4\beta2^*$ nAChRs contribute to the increased dopamine release with burst firing observed after $\alpha6\beta2^*$ nAChR blockade under control conditions. Knowledge of these changes in nAChR expression with long-term nicotine administration is critical for the development of targeted therapies with nicotinic receptor ligands for smoking cessation and neurological disorders associated with nAChR deficits such as Parkinson's disease.

The major objective of the present study was to investigate the effect of chronic nicotine treatment on nAChR-mediated dopamine release. Our results in control rats showed that evoked release was similar with single and multiple pulse stimulation, as previously shown, probably because of the development of short-term depression (Exley et al., 2008; Meyer et al., 2008). Long-term nicotine treatment led to an overall increase in evoked striatal dopamine release that was also similar with nonburst and burst firing. This functional modification with prolonged treatment may represent a long-term molecular adaptation to nicotine, because previous reports have shown that short-term nicotine exposure enhances release with burst but not with nonburst firing (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Exley et al., 2008). Our findings of enhanced release after nicotine treatment correspond with results from some in vitro [^{3}H]dopamine release assays and in vivo microdialysis studies (Rowell and Wonnacott, 1990; Marshall et al., 1997; Visanji et al., 2006), although they contrast with other work that reported a decrease (Westfall and Perry, 1986; Perry et al., 2007) or no change (Janson et al., 1991; Grilli et al., 2005). These discrepancies may relate to variations in dosing protocols, the type of preparation used, measurement of exogenous or endogenous dopamine, evaluation of nAChR-induced or stimulus-evoked release, and/or other factors.

An important question is the role of $\alpha6\beta2^*$ nAChRs in regulating striatal dopamine release with long-term nicotine dosing because this subtype makes a significant contribution

to release under control conditions (Quik and McIntosh, 2006; Exley et al., 2008; Meyer et al., 2008). Our initial studies using striatal slices from control rats showed that the selective α6β2* antagonist α-CtxMII blocked ~50% of dopamine release evoked with single pulse stimulation, consistent with recent results (Exley et al., 2008; Meyer et al., 2008). By contrast, block with α-CtxMII significantly increased release with burst firing in slices from control rats. These data at first seem distinct from those of Meyer et al. (2008), who observed no difference with nonburst and burst firing. The most likely explanation for this difference is that the α6β2* subtype plays a more prominent role in modulating dopamine release probability at lower stimulus intensity (Exley et al., 2008; Meyer et al., 2008). In the present experiments, release was done at 60% of maximum, whereas maximal stimulation was used in the aforementioned studies.

We subsequently assessed the effects of long-term nicotine treatment on nonburst- and burst-evoked dopamine release from striatal slices with and without α-CtxMII. α-CtxMII blocked ~50% of dopamine release evoked with single-pulse stimulation, similar to our results in control slices. Unexpectedly, however, burst firing elicited a pattern of release similar to that of a single pulse. Receptor studies were performed to elucidate the nAChR subtypes that may mediate these differential functional effects with long-term nicotine dosing. Administration of nicotine in the drinking water resulted in an up-regulation of α4β2* and down-regulation of α6β2* nAChRs in striatum of nicotine-treated rats. Thus, this mode of treatment to rats yielded results similar to those previously observed in mice (Lai et al., 2005; Khwaja et al., 2007).

We next investigated whether long-term nicotine treatment altered expression of the two major α6β2* subtypes present in striatum, the α6α4β2* and α6(nonα4)β2* nAChRs. The data from the rat competition studies suggested that there were differential effects of nicotine treatment on these two subtypes with a decline in the α6α4β2* nAChR subtype. Such a result was much more readily evident from our receptor competition studies in α4 nAChR knockout mice, which showed that nicotine treatment led to a loss of the α6α4β2* nAChR subtype but increased the α6(nonα4)β2* nAChR subtype. These observations in an in vivo model are consistent with previous results using transfected cell culture systems, in which nicotine exposure up-regulated either transfected α4β2 or α6(nonα4)β2 nAChRs (Kuryatov et al., 2005; Sallette et al., 2005; Tumkosit et al., 2006; Walsh et al., 2008). The present findings in wild-type mice also show that nicotine increases α4β2* nAChRs,

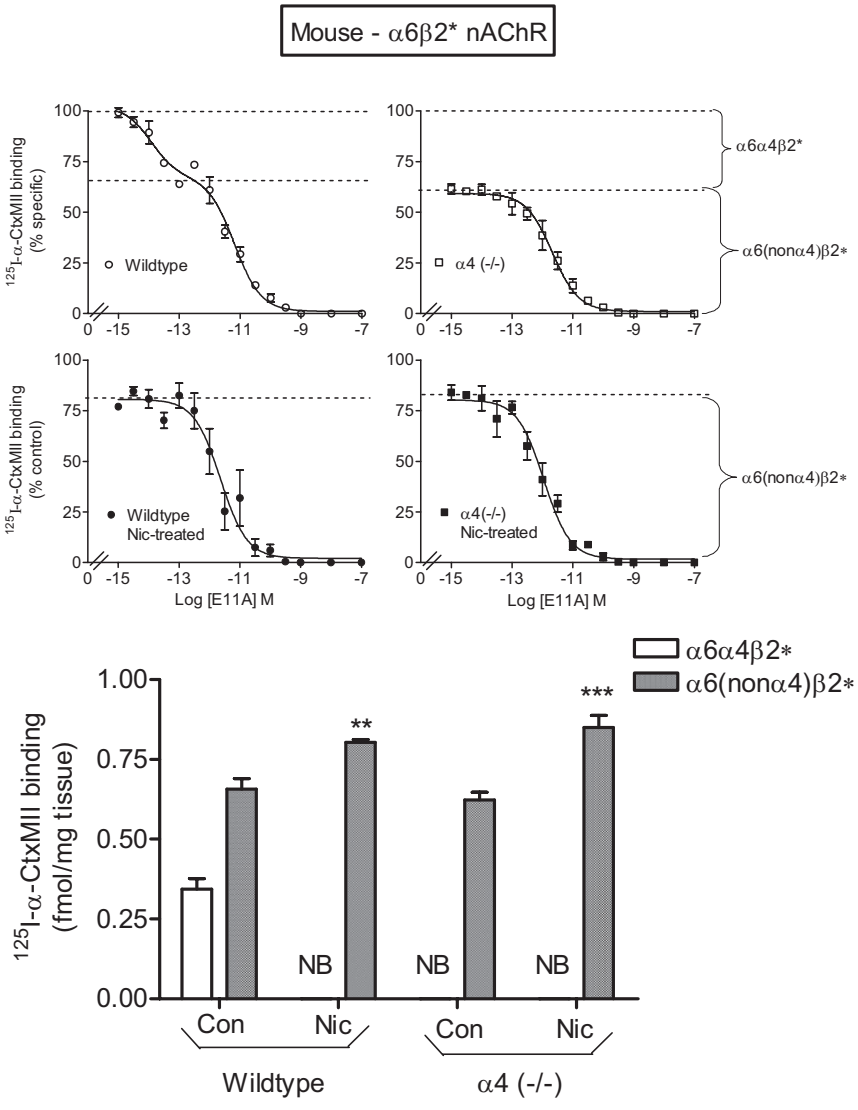

Fig. 6. Decrease in striatal α6α4β2* nAChRs but increase in α6(nonα4)β2* nAChRs with nicotine treatment. Wild-type and α4 nAChR-null mutant (−/−) mice were maintained at the final dose of nicotine in the drinking water for 14 days. Striatal ^{125}I-α-CtxMII binding was subsequently determined in the presence and absence of a range of concentrations of E11A (10^{-15}–10^{-7} M). Two site binding curves were obtained in striatum from wild-type mice, suggesting the presence of at least two α6β2* nAChR populations. In striatum of α4(−/−) mice, E11A inhibited ^{125}I-α-Ctx MII binding in a monophasic manner, as expected because α4(−/−) mice do not express α6α4β2* nAChRs. Competition analysis of E11A inhibition of ^{125}I-α-CtxMII binding in nicotine-treated wild-type and α4(−/−) mice yielded similar monophasic curves, suggesting the presence of only α6(nonα4)β2* nAChRs. This site was preferentially increased with nicotine treatment in α4(−/−) mice, as depicted in the summary graph (lower). Values represent mean ± S.E.M. of three mice. Where the SEM is not depicted, it fell within the symbol. Significance of difference from respective nontreated control using a Bonferroni post hoc test, **, $p < 0.01$; ***, $p < 0.001$. NB, no binding.

whereas studies with the $\alpha 4$ knockout mice clearly demonstrate an increase in the $\alpha 6(non\alpha 4)\beta 2^*$ population. A possible explanation for the decline in $\alpha 6\alpha 4\beta 2^*$ subtype may be that nicotine acts as a chaperone to more sensitively up-regulate $\alpha 4^*$ AChRs compared with those containing the $\alpha 6^*$ nAChRs. This would result in a nicotine-induced increase in the $\alpha 4\beta 2^*$ subtype at the expense of $\alpha 6\alpha 4\beta 2^*$ subtype when both these receptors are present simultaneously. On the other hand, nicotine exposure would still result in the increase in $\alpha 6(non\alpha 4)\beta 2^*$ nAChRs.

These findings have important implications for the functional changes observed with nicotine treatment. As mentioned earlier, $\alpha 6\beta 2^*$ nAChR-mediated dopamine release after single-pulse stimulation was decreased by ~50% in the presence of α-CtxMII in striatum of both control and nicotine-treated animals. Thus the increase in the $\alpha 6(non\alpha 4)\beta 2^*$ nAChR subtype with nicotine treatment most likely compensated for the loss of the $\alpha 6\alpha 4\beta 2^*$ nAChR subtype. By contrast, the pattern of release with burst firing after $\alpha 6\beta 2^*$ nAChR blockade was distinct in control and nicotine-treated rats. One explanation for this difference is that the $\alpha 6(non\alpha 4)\beta 2^*$ is partially responsible for alleviating the characteristic short-term depression in dopamine release. This could suggest that the $\alpha 6\alpha 4\beta 2^*$ nAChR subtype mediates the differential effects observed under control conditions because these receptors are not present after long-term nicotine treatment. Because not all receptors are necessarily functional, another possibility is that the $\alpha 6(non\alpha 4)\beta 2^*$ nAChR subtype is not involved in mediating dopamine release at higher frequencies, resulting in a similar release with nonburst- and burst-stimulated release in the nicotine-treated animals. This might imply that $\alpha 6\alpha 4\beta 2^*$ nAChRs primarily modulate dopamine release with burst firing; however, because this receptor subtype is lost with nicotine treatment, the effect of $\alpha 6\beta 2^*$ nAChR blockade is no longer observed in nicotine-treated animals.

Our studies primarily focused on $\alpha 6\beta 2^*$ nAChR-mediated dopamine release with long-term nicotine treatment. However, the results also show that nicotine treatment increased evoked striatal dopamine release in the presence of the $\alpha 6\beta 2^*$ nAChR blocker α-CtxMII, suggesting that $\alpha 4\beta 2^*$ nAChR function was increased. These data contrast with earlier reports, which identified no change in $\alpha 4\beta 2^*$ nAChR-mediated release with long-term nicotine treatment, a discrepancy that most likely relates to the different technique used to evaluate dopamine release (i.e., [^{3}H]dopamine release from synaptosomal preparations) (Lai et al., 2005; Khwaja et al., 2007; Perry et al., 2007). All together, these data suggest that alterations in both the $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs populations contribute to the changes observed in nicotine-treated animals with burst firing.

A question that arises is the relationship between the different nAChR subtypes in the striatum. All $\alpha 6\beta 2^*$ nAChRs seem to be present on dopaminergic nerve terminals because they decline in parallel with dopaminergic measures after nigrostriatal damage (Quik et al., 2001; Quik et al., 2003). However, the $\alpha 6\alpha 4\beta 2^*$ subtype is preferentially lost with moderate nigrostriatal damage, whereas the $\alpha 6(non\alpha 4)\beta 2^*$ subtype is decreased only with more severe dopaminergic damage, suggesting that the two $\alpha 6\beta 2^*$ nAChR subtypes are present on different subsets of dopaminergic neurons (Bordia et al., 2007). Evidence also indicates that the $\alpha 4\beta 2^*$ nAChR is present on yet another population of dopaminergic neurons, because lesion studies show that they are decreased only with severe nigrostriatal damage (Kulak et al., 2002). As well, their distinct functional characteristics assessed using cyclic voltammetry suggest that they are present on a unique set of dopaminergic neurons compared with the $\alpha 6\beta 2^*$ subtypes (Meyer et al., 2008).

All together, these data underscore the complexity of nAChR expression and function in the striatum and the potential for a multifaceted regulation of dopaminergic function with long-term nicotine exposure. Moreover, they suggest that long-term nicotine treatment may differentially regulate nAChR subtype expression and function by virtue of their localization in dopaminergic neuron populations that possess distinct regulatory mechanisms.

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References

Bordia T, Grady SR, McIntosh JM, and Quik M (2007) Nigrostriatal damage preferentially decreases a subpopulation of $\alpha 6\beta 2^*$ nAChRs in mouse, monkey, and Parkinson's disease striatum. *Mol Pharmacol* **72:**52–61.

Dani JA and Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu Rev Pharmacol Toxicol* **47:**699–729.

Exley R, Clements MA, Hartung H, McIntosh JM, and Cragg SJ (2008) Alpha6-containing nicotinic acetylcholine receptors dominate the nicotine control of dopamine neurotransmission in nucleus accumbens. *Neuropsychopharmacology*, in press.

Fagen ZM, Mitchum R, Vezina P, and McGehee DS (2007) Enhanced nicotinic receptor function and drug abuse vulnerability. *J Neurosci* **27:**8771–8778.

Gotti C, Moretti M, Gaimarri A, Zanardi A, Clementi F, and Zoli M (2007) Heterogeneity and complexity of native brain nicotinic receptors. *Biochem Pharmacol* **74:**1102–1111.

Grady SR, Salminen O, Laverty DC, Whiteaker P, McIntosh JM, Collins AC, and Marks MJ (2007) The subtypes of nicotinic acetylcholine receptors on dopaminergic terminals of mouse striatum. *Biochem Pharmacol* **74:**1235–1246.

Grilli M, Parodi M, Raiteri M, and Marchi M (2005) Chronic nicotine differentially affects the function of nicotinic receptor subtypes regulating neurotransmitter release. *J Neurochem* **93:**1353–1360.

Janhunen S and Ahtee L (2007) Differential nicotinic regulation of the nigrostriatal and mesolimbic dopaminergic pathways: implications for drug development. *Neurosci Biobehav Rev* **31:**287–314.

Janson AM, Meana JJ, Goiny M, and Herrera-Marschitz M (1991) Chronic nicotine treatment counteracts the decrease in extracellular neostriatal dopamine induced by a unilateral transection at the mesodiencephalic junction in rats: a microdialysis study. *Neurosci Lett* **134:**88–92.

Khwaja M, McCormack A, McIntosh JM, Di Monte DA, and Quik M (2007) Nicotine partially protects against paraquat-induced nigrostriatal damage in mice; link to alpha6beta2* nAChRs. *J Neurochem* **100:**180–190.

Kulak JM, McIntosh JM, and Quik M (2002) Loss of nicotinic receptors in monkey striatum after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment is due to a decline in α-conotoxin MII sites. *Mol Pharmacol* **61:**230–238.

Kuryatov A, Luo J, Cooper J, and Lindstrom J (2005) Nicotine acts as a pharmacological chaperone to up-regulate human $\alpha 4\beta 2$ acetylcholine receptors. *Mol Pharmacol* **68:**1839–1851.

Lai A, Parameswaran N, Khwaja M, Whiteaker P, Lindstrom JM, Fan H, McIntosh JM, Grady SR, and Quik M (2005) Long-term nicotine treatment decreases striatal alpha 6* nicotinic acetylcholine receptor sites and function in mice. *Mol Pharmacol* **67:**1639–1647.

Mao D, Perry DC, Yasuda RP, Wolfe BB, and Kellar KJ (2008) The alpha4beta2alpha5 nicotinic cholinergic receptor in rat brain is resistant to up-regulation by nicotine in vivo. *J Neurochem* **104:**446–456.

Marshall DL, Redfern PH, and Wonnacott S (1997) Presynaptic nicotinic modulation of dopamine release in the three ascending pathways studied by in vivo microdialysis: comparison of naive and chronic nicotine-treated rats. *J Neurochem* **68:**1511–1519.

Marubio LM, Gardier AM, Durier S, David D, Klink R, Arroyo-Jimenez MM, McIntosh JM, Rossi F, Champtiaux N, Zoli M, et al. (2003) Effects of nicotine in the dopaminergic system of mice lacking the alpha4 subunit of neuronal nicotinic acetylcholine receptors. *Eur J Neurosci* **17:**1329–1337.

Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, Evrard A, Cazala P, Cormier A, Mameli-Engvall M, et al. (2005) Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature* **436:**103–107.

McCallum SE, Parameswaran N, Bordia T, Fan H, Tyndale RF, Langston JW, McIntosh JM, and Quik M (2006) Increases in alpha4* but not alpha3*/alpha6* nicotinic receptor sites and function in the primate striatum following chronic oral nicotine treatment. *J Neurochem* **96:**1028–1041.

Meyer EL, Yoshikami D, and McIntosh JM (2008) The neuronal nicotinic acetylcholine receptors alpha4 and alpha6 differentially modulate dopamine release in mouse striatal slices. *J Neurochem* **105:**1761–1769.

Perez XA, Parameswaran N, Huang LZ, O'Leary KT, and Quik M (2008) Presynaptic

dopaminergic compensation after moderate nigrostriatal damage in nonhuman primates. *J Neurochem* **105:**1861–1872.
Perry DC, Mao D, Gold AB, McIntosh JM, Pezzullo JC, and Kellar KJ (2007) Chronic nicotine differentially regulates α6- and β3-containing nicotinic cholinergic receptors in rat brain. *J Pharmacol Exp Ther* **322:**306–315.
Pietilä K and Ahtee L (2000) Chronic nicotine administration in the drinking water affects the striatal dopamine in mice. *Pharmacol Biochem Behav* **66:**95–103.
Quik M, Bordia T, and O'Leary K (2007) Nicotinic receptors as CNS targets for Parkinson's disease. *Biochem Pharmacol* **74:**1224–1234.
Quik M, Bordia T, Okihara M, Fan H, Marks MJ, McIntosh JM, and Whiteaker P (2003) L-DOPA treatment modulates nicotinic receptors in monkey striatum. *Mol Pharmacol* **64:**619–628.
Quik M and McIntosh JM (2006) Striatal α6* nicotinic acetylcholine receptors: potential targets for Parkinson's disease therapy. *J Pharmacol Exp Ther* **316:**481–489.
Quik M, Polonskaya Y, Kulak JM, and McIntosh JM (2001) Vulnerability of 125I-alpha-conotoxin MII binding sites to nigrostriatal damage in monkey. *J Neurosci* **21:**5494–5500.
Rice ME and Cragg SJ (2004) Nicotine amplifies reward-related dopamine signals in striatum. *Nat Neurosci* **7:**583–584.
Ross SA, Wong JY, Clifford JJ, Kinsella A, Massalas JS, Horne MK, Scheffer IE, Kola I, Waddington JL, Berkovic SF, et al. (2000) Phenotypic characterization of an alpha 4 neuronal nicotinic acetylcholine receptor subunit knock-out mouse. *J Neurosci* **20:**6431–6441.
Rowell PP and Wonnacott S (1990) Evidence for functional activity of up-regulated nicotine binding sites in rat striatal synaptosomes. *J Neurochem* **55:**2105–2110.
Sallette J, Pons S, Devillers-Thiery A, Soudant M, Prado de Carvalho L, Changeux JP, and Corringer PJ (2005) Nicotine upregulates its own receptors through enhanced intracellular maturation. *Neuron* **46:**595–607.
Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC, and Grady SR (2004) Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Mol Pharmacol* **65:**1526–1535.
Singh N, Pillay V, and Choonara YE (2007) Advances in the treatment of Parkinson's disease. *Prog Neurobiol* **81:**29–44.
Tapper AR, McKinney SL, Marks MJ, and Lester HA (2007) Nicotine responses in hypersensitive and knockout alpha 4 mice account for tolerance to both hypothermia and locomotor suppression in wild-type mice. *Physiol Genomics* **31:**422–428.
Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, and Lester HA (2004) Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. *Science* **306:**1029–1032.
Tumkosit P, Kuryatov A, Luo J, and Lindstrom J (2006) β3 subunits promote expression and nicotine-induced up-regulation of human nicotinic α6* nicotinic acetylcholine receptors expressed in transfected cell lines. *Mol Pharmacol* **70:**1358–1368.
Visanji NP, Mitchell SN, O'Neill MJ, and Duty S (2006) Chronic pre-treatment with nicotine enhances nicotine-evoked striatal dopamine release and alpha6 and beta3 nicotinic acetylcholine receptor subunit mRNA in the substantia nigra pars compacta of the rat. *Neuropharmacology* **50:**36–46.
Walsh H, Govind AP, Mastro R, Hoda JC, Bertrand D, Vallejo Y, and Green WN (2008) Up-regulation of nicotinic receptors by nicotine varies with receptor subtype. *J Biol Chem* **283:**6022–6032.
Westfall TC and Perry H (1986) The nicotinic-induced release of endogenous dopamine from rat striatal slices from animals chronically exposed to dimethylphenylpiperazinium (DMPP). *Neurosci Lett* **71:**340–344.
Whiteaker P, McIntosh JM, Luo S, Collins AC, and Marks MJ (2000) ^{125}I-α-conotoxin MII identifies a novel nicotinic acetylcholine receptor population in mouse brain. *Mol Pharmacol* **57:**913–925.
Wonnacott S, Sidhpura N, and Balfour DJ (2005) Nicotine: from molecular mechanisms to behaviour. *Curr Opin Pharmacol* **5:**53–59.
Zhang H and Sulzer D (2004) Frequency-dependent modulation of dopamine release by nicotine. *Nat Neurosci* **7:**581–582.
Zhou FM, Liang Y, and Dani JA (2001) Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nat Neurosci* **4:**1224–1229.
Zhou FM, Wilson CJ, and Dani JA (2002) Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J Neurobiol* **53:**590–605.

Address correspondence to: Maryka Quik, The Parkinson's Institute, 675 Almanor Avenue, Sunnyvale, CA 94085. E-mail: mquik@parkinsonsinstitute.org