

Nigrostriatal Damage Preferentially Decreases a Subpopulation of $\alpha 6\beta 2^*$ nAChRs in Mouse, Monkey, and Parkinson's Disease Striatum

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ABSTRACT

Parkinson's disease is a neurodegenerative movement disorder characterized by a loss of substantia nigra dopamine neurons, and corresponding declines in molecular components present on striatal dopaminergic nerve terminals. These include the $\alpha 6\beta 2^*$ nicotinic acetylcholine receptors (nAChRs), which are localized exclusively on dopamine terminals in striatum (*denotes the presence of possible additional subunits). In this study, we used a novel α -conotoxin MII (α -CtxMII) analog E11A to further investigate $\alpha 6\beta 2^*$ nAChR subtypes in mouse, monkey, and human striatum. Receptor competition studies with ^{125}I - α -CtxMII showed that E11A inhibition curves were biphasic, suggesting the presence of two distinct $\alpha 6\beta 2^*$ nAChR subtypes. These include a very high (femtomolar) and a high (picomolar) affinity site, with ~40% of the sites in the very high affinity form. It is noteworthy that only the high-affinity form was

detected in $\alpha 4$ nAChR-null mutant mice. Because ^{125}I - α -CtxMII binds primarily to $\alpha 6\alpha 4\beta 2\beta 3$ and $\alpha 6\beta 2\beta 3$ nAChR subtypes in mouse striatum, these data suggest that the population lost in the $\alpha 4$ knockout mice was the $\alpha 6\alpha 4\beta 2\beta 3$ subtype. We next investigated the effect of nigrostriatal lesioning on these two striatal $\alpha 6\beta 2^*$ populations in two animal models and in Parkinson's disease. There was a preferential loss of the very high affinity subtype in striatum of mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), monkeys treated with MPTP, and patients with Parkinson's disease. These data suggest that dopaminergic terminals expressing the $\alpha 6\alpha 4\beta 2\beta 3$ population are selectively vulnerable to nigrostriatal damage. This latter nAChR subtype, identified with α -CtxMII E11A, may therefore provide a unique marker for dopaminergic terminals particularly sensitive to nigrostriatal degeneration in Parkinson's disease.

Multiple nicotinic acetylcholine receptors (nAChRs), including the $\alpha 4\beta 2^*$ (* denotes nicotinic receptors containing the indicated α and/or β subunit and possible additional subunits) and $\alpha 6\beta 2^*$ subtypes, are present in striatum (Wonnacott et al., 2005; Gotti et al., 2006c), a region of particular relevance to Parkinson's disease. This neurodegenerative movement disorder is characterized by declines in dopaminergic cell bodies in the substantia nigra and nerve terminals in the striatum (Davidson et al., 1971; Hornykiewicz, 1975; Olanow, 2004; Samii et al., 2004; Savitt et al., 2006). Because nAChRs are present on striatal dopaminergic terminals, these receptors are correspondingly decreased in experimental models of nigrostriatal damage and Parkinson's disease (Quik, 2004). This includes the $\alpha 6\beta 2^*$ nAChR that is local-

ized exclusively on nigrostriatal dopamine neurons and also the $\alpha 4\beta 2^*$ receptor present both on dopamine nerve terminals and other striatal neurons (Zoli et al., 2002; Champiaux et al., 2003; Quik et al., 2005; Salminen et al., 2005; Gotti et al., 2006a). Declines in $\alpha 6\beta 2^*$ nAChRs with nigrostriatal damage closely parallel losses in dopaminergic markers (Quik et al., 2001). In contrast, $\alpha 4\beta 2^*$ receptors are also decreased but only with severe nigrostriatal damage (Kulak et al., 2002a). These findings raised the question of whether nerve terminals expressing $\alpha 6\beta 2^*$ nAChRs are more susceptible to nigrostriatal degeneration, suggesting that these receptors represent a marker for such dopaminergic neurons. However, no conclusive relationship has yet been observed using existing nAChR ligands.

Radioligands currently available to study striatal $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR expression include: epibatidine (which labels $\alpha 2^*$ - $\alpha 6^*$ nAChRs), A85380 (which identifies $\beta 2^*$ nAChRs), and α -conotoxin MII (α -CtxMII, which interacts with $\alpha 3\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs) (Gotti and Clementi, 2004; Quik, 2004; Quik and McIntosh, 2006). Because mice and

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ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; α -CtxMII, α -conotoxin MII; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; RTI-121, 3 β -(4-iodophenyl)tropane-2 β -carboxylic acid; BSA, bovine serum albumin.

rats do not express $\alpha 3\beta 2^*$ nAChRs in striatum, ^{125}I - α -CtxMII has proved an excellent tool to label $\alpha 6\beta 2^*$ nAChRs in rodents (Whiteaker et al., 2002; Zoli et al., 2002; Champtiaux et al., 2003). However, studies in monkey and human striatum are more complex because of the presence of both the $\alpha 6\beta 2^*$ and $\alpha 3\beta 2^*$ subtypes (Quik et al., 2005; Gotti et al., 2006a). A search for more selective agents that discriminate between these subtypes led to the development of α -CtxMII E11A, in which the glutamic acid at position 11 is replaced with alanine (McIntosh et al., 2004). Oocyte expression studies showed that E11A blocked chimeric $\alpha 6/\alpha 3\beta 2$ receptors with ~ 50 -fold lower IC_{50} than $\alpha 3\beta 2$ nAChRs (McIntosh et al., 2004). These data suggested that the use of E11A together with ^{125}I - α -CtxMII may allow for a clearer measure of $\alpha 6\beta 2^*$ nAChR subtypes in monkey and human striatum.

Our objective was therefore to investigate striatal $\alpha 6\beta 2^*$ nAChR expression using this novel α -CtxMII analog. We were surprised to observe that E11A discriminated between a very-high and high-affinity $\alpha 6\beta 2^*$ nAChR population in mouse striatum. Further studies also demonstrated the presence of two $\alpha 6\beta 2^*$ nAChR populations in striatum of monkeys and humans. We next investigated how these subtypes were altered with nigrostriatal damage. The results of lesion studies, coupled with experiments using $\alpha 4$ nAChR-null mutant mice, suggest there is a preferential loss of the $\alpha 6\alpha 4\beta 2\beta 3$ compared with the $\alpha 6\beta 2\beta 3$ nAChR subtype with nigrostriatal damage.

Materials and Methods

Mouse Treatment. Ten- to 12-week old male C57BL/6 mice were purchased from Simonsen Laboratories (Gilroy, CA). They were housed in a temperature-controlled room with a 13-h/11-h light/dark cycle in groups of three or four per cage and had free access to food and water. Four days after arrival, unlesioned mice were administered saline. Lesioned mice were given MPTP (10 mg/kg every 2 h three to four times for 1 day, or 10 to 20 mg/kg twice weekly for 3 weeks) to generate animals with varying $\alpha 6\beta 2^*$ nAChR loss. Mice were killed by cervical dislocation 7 days after the last MPTP or saline injection. The brains were removed, quickly frozen in isopentane on dry ice, and stored at -80°C . When required, they were equilibrated to -15°C and 14- μm sections prepared using a cryostat. The sections were thaw-mounted onto Superfrost Plus slides (Fisher, Pittsburgh, PA) air-dried and stored at -80°C for autoradiography.

All procedures used conform to the Institute of Laboratory Animal Resources (1996) guidelines and were approved by the Institutional Animal care and use committee. All efforts were made to minimize the number and suffering of animals used.

The $\alpha 4$ -null mutant mice, originally obtained from the laboratory of Dr. John Drago (Ross et al., 2000), were bred and maintained at the Institute for Behavioral Genetics, University of Colorado (Boulder, CO). All care and procedures were in accordance with guidelines and approval of the Animal Care and Utilization Committee of the University of Colorado, Boulder. Mice were weaned at 25 days of age and housed with same-sex littermates. A 12-h light/dark cycle was used, with room temperature at 22°C . Mice had free access to food and water. DNA was extracted from tail clippings, taken at 40 days of age, using the DNeasy kit from QIAGEN (Valencia, CA), and analyzed by polymerase chain reaction for genotype (Salminen et al., 2004). Mice for this study were of a mixed genetic background; wild-type and null mutants were littermates and age matched (~ 250 days old). Mice were sacrificed by cervical dislocation, and the brains removed and quickly frozen in isopentane on dry ice at -30°C and stored at -80°C . Sections were subsequently prepared for autoradiography.

Monkey Treatment. Adult female squirrel monkeys (*Saimiri sciureus*) weighing between 0.5 to 0.7 kg were purchased from Osage Research Primates (Osage Beach, MO) and from the Primate Research Laboratory (University of South Alabama, Mobile, AL). Immediately after arrival, monkeys were quarantined for 1 month according to California State regulations. They were housed in a room with a 13-h/11-h light/dark cycle and given food once daily with water ad libitum. After quarantine, the monkeys were treated with saline ($n = 6$) or MPTP ($n = 8$). MPTP was injected subcutaneously at a dose of 1.5 to 2.0 mg/kg once monthly from one to three times for cumulative doses of 3.0 to 5.6 mg/kg. Parkinsonism was rated during week 4 after MPTP injection using a modified Parkinson rating scale for the squirrel monkey (Langston et al., 2000). The disability scores ranged from 0 to 24 for a severely parkinsonian animal. The composite score was evaluated by an assessment of 1) spatial hypokinesia (reduction in use of the available cage space), 2) body bradykinesia (increased slowness in body movement), 3) manual dexterity (left and right), 4) balance, and 5) freezing. Each parameter was evaluated using a five-point scale, 0 being normal. The monkeys in the lesion 1 group had a score of 5.5 ± 3.2 ; those in the lesion 2 group had a score of 9.6 ± 1.9 . The monkeys were euthanized ~ 1 month after the last MPTP injection according to the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. This was done by i.p. injection of 1.5 ml of euthanasia solution (390 mg/ml sodium pentobarbital and 50 mg/ml phenytoin sodium), followed by 1.5 ml/kg of the same solution administered i.v.. All

TABLE 1

Demographics of the human cases

Tissue was obtained from the brain bank at the Parkinson's Institute and the Institute for Aging and Dementia at the University of California Irvine. An "Ever" smoking status indicates individuals who smoked during their lifetime, whereas "Never" indicates those who never smoked. There was no significant difference in age or post mortem delay between the two groups.

Group & Case	Smoking history	Age years	Sex	Postmortem h	Anti-Parkinsonian Medication
Control cases					
1	N.A.	78	F	14	
2	N.A.	72	F	9	
3	N.A.	74	F	13.5	
4	N.A.	70	M	7.5	
5	Ever	55	M	13	
Mean \pm S.E.M.		70 ± 4		11 ± 1	
Parkinson's disease					
1	Never	67	F	21	L-DOPA/carbidopa
2	Never	83	M	5	L-DOPA/carbidopa
3	Ever	84	M	6	L-DOPA/carbidopa/pramipexole, donepezil
4	Ever	77	M	4	N.A.
Mean \pm S.E.M.		78 ± 4		9 ± 4	

N.A., not available.

studies were performed according to Institute of Laboratory Animal Resources (1996) guidelines, and were approved by the Institutional Animal Care and Use Committee at the Parkinson's Institute.

The brains were then removed, rinsed in saline, and divided along the midline. Half of the brain was placed in a mold and cut into 6-mm blocks. These were immediately frozen in isopentane on dry ice and stored at -80°C . Sections ($20\text{ }\mu\text{m}$) were cut from these blocks using a cryostat, mounted on Superfrost Plus slides, air-dried, and stored at -80°C for further use. The squirrel monkey atlas (Emmers and Akert, 1963) was used to identify brain regions. The level of sectioning ranged from A12.0 to A9.5. A previous study from our laboratory had shown that there were no significant anterior-posterior differences in nicotinic receptor or dopamine transporter expression in

control or MPTP-lesioned squirrel monkey, in contrast to well documented medial to lateral gradients (Quik et al., 2002).

Human Brain Tissue. Parkinson's disease ($n = 4$) and control samples ($n = 5$) were obtained from the brain bank at the Parkinson's Institute and from the Institute for Brain Aging and Dementia at the University of California, Irvine (Table 1). The control samples had no known clinical history of neurological or psychiatric disease and showed no evidence of neuropathology and/or cell loss in the substantia nigra. There was no Alzheimer-type or cerebrovascular pathology. Clinical assessment for Parkinson's disease patients included standard clinical criteria and was confirmed at autopsy by the presence of Lewy bodies and loss of pigmented neurons in the substantia nigra. The subjects were age-matched with a mean age of 78 ± 4 years for the Parkinson's disease and 70 ± 4 for the control cases. Post mortem delays were similar in the control and Parkinson's disease cases. For the tissue obtained from The Parkinson's Institute Brain Bank, the brain was collected at autopsy, and tissue blocks containing the caudate and putamen dissected and immediately frozen on a glass slide in isopentane on dry ice. They were then stored at -80°C . Tissue from the Institute for Brain and Aging and Dementia (University of California, Irvine, CA) was shipped as frozen blocks on dry ice. When required, sections ($20\text{ }\mu\text{m}$) were cut on a cryostat at -15°C , thaw-mounted on Superfrost Plus slides, and stored at -80°C .

^{125}I -RTI-121 Autoradiography. Binding of ^{125}I -RTI-121 (2200 Ci/mmol; PerkinElmer Life and Analytical Sciences, Boston, MA) was used to evaluate the dopamine transporter. Striatal brain sections were initially incubated in buffer, pH 7.4, containing 50 mM Tris-HCl, 120 mM NaCl, and 5 mM KCl, twice for 15 min. The sections were then incubated for 2 h in the same buffer also containing 0.025% BSA, 1 μM fluoxetine, and 50 pM ^{125}I -RTI-121. The sections were washed four times for 15 min each time at 4°C in preincubation buffer and once in ice-cold water. They were then air-dried and placed against Kodak MR film (PerkinElmer Life and Analytical Sciences) for 1 to 3 days with ^{125}I -microscale standards (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK). Nomifensin (100 μM) was used to define nonspecific binding.

^{125}I - α -CtxMII Autoradiography. ^{125}I - α -CtxMII (specific activity, 2200 Ci/mmol) was prepared as described previously (Whiteaker et al., 2000), and binding was performed as reported previously (Quik et al., 2001, 2004). Competition studies (1.0 fM–0.1 μM) were done using α -CtxMII or α -CtxMII E11A, which was prepared according to a previous report (McIntosh et al., 2004). 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% BSA, pH 7.5) plus 1 mM phenylmethylsulfonyl fluoride. This was followed by a 1-h incubation at room temperature in binding buffer plus 0.5% BSA, also containing 5 mM EDTA, 5 mM EGTA, and 10 $\mu\text{g/ml}$ each of aprotinin, leupeptin, pepstatin A, and 0.5 nM ^{125}I - α -CtxMII. To terminate the assay, slides were washed for 10 min

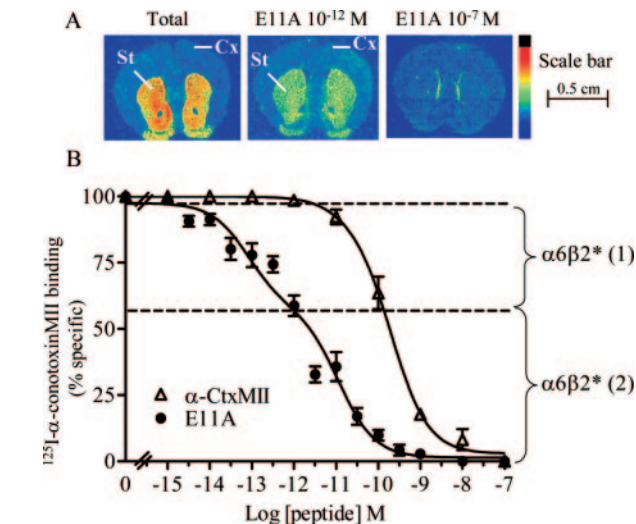


Fig. 1. α -CtxMII E11A discriminates between two $\alpha 6\beta 2^*$ nAChR populations in unlesioned mouse striatum, whereas native α -CtxMII does not. Striatal sections were incubated with 0.5 nM ^{125}I - α -CtxMII in the absence and presence of the indicated concentrations of either native α -CtxMII or the analog E11A. A, autoradiographic images depicting partial (10^{-12} M) and complete (10^{-7} M) displacement of ^{125}I - α -CtxMII binding (Total) by E11A in unlesioned mouse striatum. Background binding was similar to that obtained with 10^{-4} M nicotine. B, biphasic inhibition of ^{125}I - α -CtxMII binding by E11A in unlesioned mouse striatum. α -CtxMII E11A discriminates between at least two $\alpha 6\beta 2^*$ subtypes, very-high-affinity and high-affinity sites (data fit best to a two-site model). In contrast, monophasic inhibition of ^{125}I - α -CtxMII binding was observed in the presence of varying concentrations of unlabeled α -CtxMII (data fit best to a one-site model), suggesting that native α -CtxMII does not distinguish between the different $\alpha 6\beta 2^*$ subtypes. The color bar indicates increasing image intensity in the sequence blue, yellow, red, and black. Symbols represent the mean \pm S.E.M. of four to eight mice per group. Where the S.E.M. is not depicted, it fell within the symbol. Cx, cortex; St, striatum.

TABLE 2

Loss of the very high $\alpha 6\beta 2^*$ nAChR subtype in striata of $\alpha 4$ nAChR-null mutant mice and striata of severely lesioned mice

Results of ^{125}I - α -CtxMII competition studies by E11A in striatum from wild-type and $\alpha 4$ nAChR-null mutant mice ($-/-$) (experiment 1). Biphasic inhibition curves were obtained using striatum from wild-type mice, with monophasic curves using tissue from $\alpha 4$ nAChR-null mutant mice. The results in experiment 2 are from mice injected with saline or MPTP. The MPTP-lesioned mice were divided into two groups based on the extent of loss of striatal ^{125}I - α -CtxMII binding sites (lesion 1 and lesion 2). Biphasic inhibition curves were observed for unlesioned mice, and mice with less severe declines in ^{125}I - α -CtxMII binding (lesion 1). In contrast, monophasic curves were obtained using striatum from severely lesioned mice (lesion 2). Each value represents the means \pm S.E.M. of four to eight mice. Numbers in parentheses are the 95% confidence intervals (CI).

Experiment	Group	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			%	
1	Wild-type	Two	0.029 (0.013–0.061)	17 (10–29)	590	43	57
	$\alpha 4^{-/-}$	One		1.2 (0.58–2.5)			100
2	Mouse-unlesioned	Two	0.12 (0.085–0.18)	13 (10–17)	110	43	57
	Mouse-lesion 1	Two	0.14 (0.11–0.18)	14 (12–15)	100	35	65
	Mouse-lesion 2	One		1.5 (1.4–1.7)			100

in buffer at room temperature followed by 10 min in ice-cold binding buffer, twice for 10 min each in $0.1\times$ buffer (0°C), and two 5-s washes in water (0°C). The sections were air-dried and exposed to Kodak MR film (PerkinElmer Life Sciences, Boston, MA) for 2 to 5 days together with ^{125}I -standards (GE Healthcare). Nicotine ($100\ \mu\text{M}$) was used to determine nonspecific binding.

Data Analysis. Quantitation of optical densities for different brain areas was done using an ImageQuant system (GE Healthcare). To obtain the optical density reading for any specific neuroanatomical region, the entire area was quantitated for each species to avoid sampling error. Specifically this included the striatum for mice; the medial caudate, lateral caudate, ventral putamen, and dorsal putamen for monkeys; and caudate and putamen for the human tissue. The optical densities were determined by subtracting background from tissue values and converted to femtomoles per milligram of tissue using standard curves generated from radioactive standards simultaneously exposed to the films. Sample optical density readings were within the linear range of the film. Two to six tissue sections from any one animal or human case were used for each point in the competition curves. All values depicted in the competition curves represent the combined data from four to eight animals or human cases, as indicated in the figure legends. For the dopamine transporter studies, the binding value represents the mean of two to six adjacent sections for each animal or human case. All values are expressed as mean \pm S.E.M. of four to eight animals or human cases. Statistical comparisons were done with Prism (GraphPad, San Diego, CA) using one-way analysis of variance followed by Bonferroni post hoc test. ^{125}I - α -CtXMII competition curves were computed using GraphPad Prism. Values were considered statistically significant when $p < 0.05$.

Results

α -CtXMII E11A, but Not Native α -CtXMII, Discriminates between $\alpha 6\beta 2^*$ nAChR Populations in Unlesioned Mouse Striatum. ^{125}I - α -CtXMII competition experiments (Fig. 1) were performed using varying concentrations of unlabeled α -CtXMII ($1.0\ \text{fM}$ to $0.1\ \mu\text{M}$). The data yielded a curve that best fit to one site with an $\text{IC}_{50} = 172\ \text{pM}$ (Fig. 1B). Studies were then done with α -CtXMII E11A, which, at maximal concentrations, completely inhibited ^{125}I - α -CtXMII binding, similar to native α -CtXMII (Fig. 1B). E11A inhibition of ^{125}I - α -CtXMII in unlesioned striatum yielded a two-site inhibition curve with a hundredfold difference in affinity between sites (Fig. 1B; Table 2). The very-high-affinity site represented 43% of the total E11A-sensitive ^{125}I - α -CtXMII binding sites (Table 2). These combined data suggest that the α -CtXMII analog E11A distinguishes between two different $\alpha 6\beta 2^*$ forms in unlesioned mouse striatum, whereas α -CtXMII does not.

Preferential Loss of the Very-High-Affinity $\alpha 6\beta 2^*$ nAChR Subtype in Striatum from $\alpha 4$ nAChR Null Mutant Mice. Previous studies had suggested that there were two major $\alpha 6\beta 2^*$ nAChR in mouse striatum, the $\alpha 6\alpha 4\beta 2\beta 3$ and $\alpha 6\beta 2\beta 3$ subtypes (Salminen et al., 2004). As an approach to investigate whether these may be the ones distinguished by E11A, ^{125}I - α -CtXMII binding was done with varying concentrations of E11A using striatal tissue from $\alpha 4$ nAChR-null mutant mice that do not express the $\alpha 6\alpha 4\beta 2\beta 3$ nAChR subtype. In contrast to the biphasic ^{125}I - α -CtXMII competition curves obtained with E11A using striatum from wild-type mice, monophasic curves were observed in striatum of $\alpha 4$ nAChR-null mutant mice (Fig. 2A, Table 2). There seemed to be a preferential loss of the very high affinity component (possibly $\alpha 6\alpha 4\beta 2\beta 3$ receptors), with the residual receptor

sites more closely resembling the high-affinity site (possibly $\alpha 6\beta 2\beta 3$ receptors) in wild-type mice.

Preferential Decline in the Very-High-Affinity $\alpha 6\beta 2^*$ nAChR Subtype in MPTP-Lesioned Mouse Striatum. To evaluate the extent of nigrostriatal damage, dopamine transporter binding was done on sections from control and MPTP-treated mice (Table 3). Because some mice were less severely lesioned than others, the data from the animals

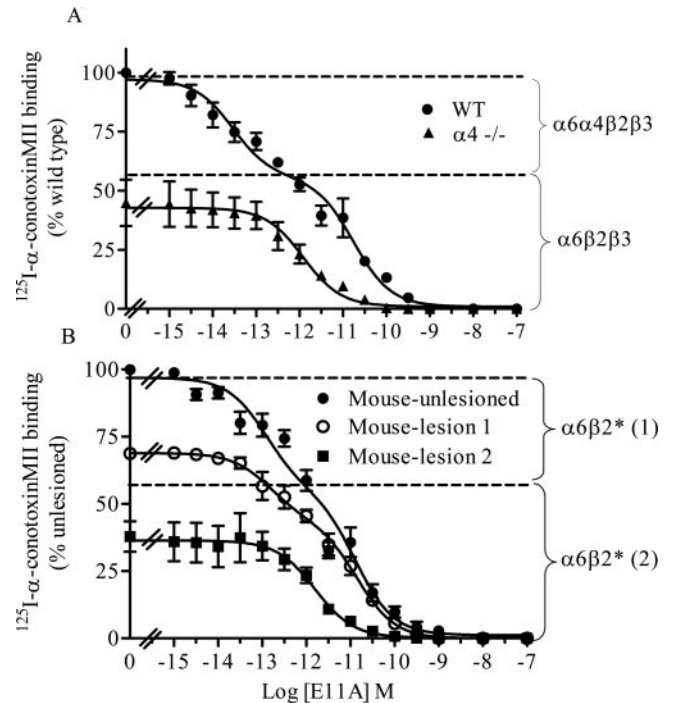


Fig. 2. Preferential loss of the very high $\alpha 6\beta 2^*$ nAChR subtype in striatum of $\alpha 4$ nAChR-null mutant mice and in striatum of severely lesioned mice. ^{125}I - α -CtXMII competition curves in the presence of varying concentrations of E11A from wild-type (WT) and $\alpha 4$ nAChR-null mutant (—/—) mice are depicted in A. Biphasic inhibition curves were obtained using striatum from wild-type mice, but monophasic inhibition using striatum from $\alpha 4$ knockout mice. B, mice were injected with saline or MPTP as described under *Materials and Methods*. The MPTP-lesioned mice were divided into two groups based on the extent of loss of striatal ^{125}I - α -CtXMII binding (lesion 1 and lesion 2). Note that with the larger MPTP lesion (lesion 2), the data are best fit to a one-site model. In contrast, biphasic inhibition of ^{125}I - α -CtXMII binding was observed with the less severe MPTP lesion (lesion 1). Symbols represent the mean \pm S.E.M. of four to eight mice per group. Where the S.E.M. is not depicted, it fell within the symbol.

TABLE 3

Decrease in striatal dopamine transporter in striatum of MPTP-treated mice and monkeys

^{125}I -RTI-121 binding was done as described under *Materials and Methods*. Animals were divided into two groups based on the extent of nigrostriatal damage, with the more severe damage in the lesion 2 group. Each value represents mean \pm S.E.M. of four to eight mice per group or four to six monkeys per group.

Species	Region	^{125}I -RTI-121		
		Control	Lesion 1	Lesion 2
		fmol/mg tissue		
Mouse	Striatum	7.72 ± 0.16	$3.19 \pm 0.47^{**}$	$0.82 \pm 0.15^{**}$
Monkey	Medial caudate	12.1 ± 0.68	$7.89 \pm 1.09^{**}$	$5.43 \pm 0.57^{**}$
	Lateral caudate	10.6 ± 0.75	$5.62 \pm 1.16^{**}$	$1.96 \pm 0.38^{**}$
	Ventral putamen	11.5 ± 0.65	$8.75 \pm 1.53^{**}$	$5.30 \pm 1.03^{**}$
	Dorsal putamen	10.6 ± 0.68	$4.09 \pm 0.75^{**}$	$1.80 \pm 0.42^{**}$

Significance of difference from control using a Bonferroni post hoc test: **, $P < 0.01$.

was divided into two groups, those with moderate (mouse-lesion 1) and those with more severe (mouse-lesion 2) nigrostriatal degeneration (Table 3). The ¹²⁵I-α-CtxMII binding values for the mice in these two groups were 69 ± 0.4% (*n* = 4) and 38 ± 5.6% (*n* = 5), respectively, compared with unlesioned mice (Fig. 2B). These declines in ¹²⁵I-α-CtxMII binding correlated well with those in the dopamine transporter (*r* = 0.93, *p* < 0.001), consistent with a previous report (Quik et al., 2003). ¹²⁵I-α-CtxMII binding values in unlesioned mice were 3.15 ± 0.06 fmol/mg of tissue (*n* = 8).

To determine how lesioning affected the two α6β2* binding components defined with E11A, ¹²⁵I-α-CtxMII binding was

done in the presence of varying concentrations of the analog in striatal tissue from MPTP-treated mice (Fig. 2B). In the mouse-lesion 1 group, E11A inhibited ¹²⁵I-α-CtxMII binding in a biphasic manner similar to that in unlesioned mice, yielding very-high-affinity (IC₅₀1 = 0.14 pM) and high-affinity (IC₅₀2 = 14 pM) binding sites (Fig. 2B; Table 2). With more severe lesioning, the two binding sites could no longer be distinguished with E11A, with the IC₅₀ intermediate between that of the original very-high-affinity and high-affinity binding sites.

α-CtxMII E11A Discriminates between α6β2* nAChR Subtypes in Unlesioned Monkey Striatum. We next in-

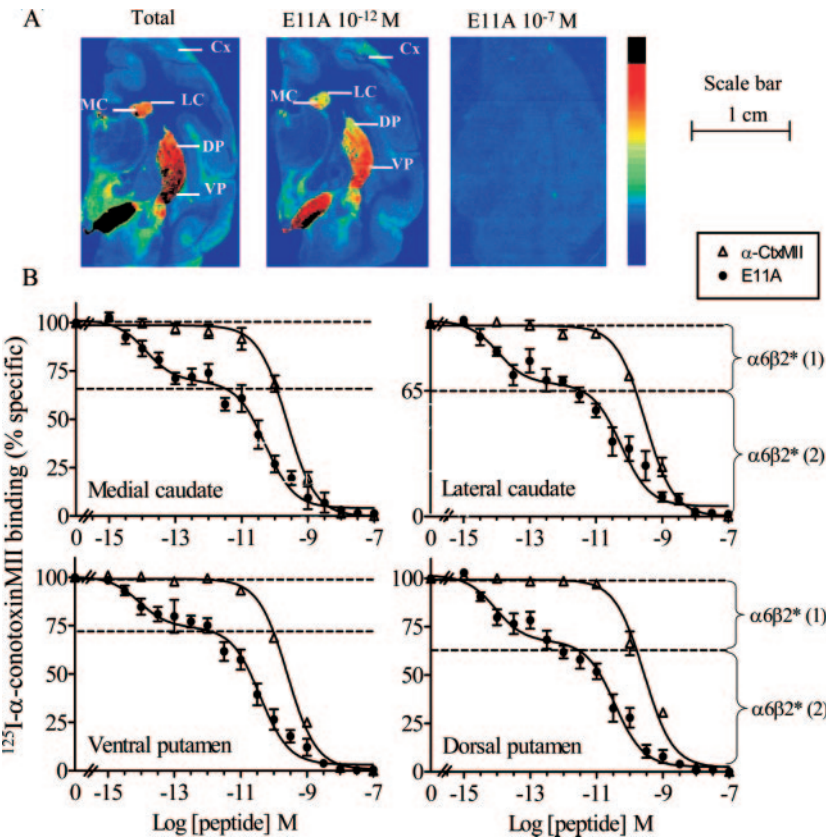


Fig. 3. α-CtxMII E11A, but not native α-CtxMII, discriminates between two α6β2* nAChR subtypes in unlesioned monkey striatum. Unlabeled α-CtxMII and the analog E11A were assessed for their ability to displace ¹²⁵I-α-CtxMII binding in four striatal areas. A, autoradiographic images depicting partial (10⁻¹² M) and complete (10⁻⁷ M) displacement of ¹²⁵I-α-CtxMII binding (Total) by E11A in unlesioned monkey striatum. Background binding was similar to that obtained with 10⁻⁴ M nicotine. B, native α-CtxMII displaced ¹²⁵I-α-CtxMII binding in a monophasic manner in unlesioned medial caudate (data fit best to a one-site model), with similar results in lateral caudate, ventral putamen, and dorsal putamen. In contrast, two-site competition curves were obtained with E11A. This suggests the presence of at least two α6β2* nAChR populations, one very high affinity and the other high affinity. The color bar indicates increasing image intensity in the sequence blue, yellow, red, and black. Symbols represent the mean ± S.E.M. of six unlesioned monkeys. Where the S.E.M. is not depicted, it fell within the symbol. Cx, cortex; DP, dorsal putamen; MC, medial caudate; LC, lateral caudate; VP, ventral putamen.

TABLE 4
E11A discriminates between α6β2* nAChR subtypes in unlesioned monkey striatum

Competition analyses of ¹²⁵I-α-CtxMII binding by E11A in unlesioned monkey striatum yielded inhibition curves that best fit to a two-site model, whereas competition with native unlabeled α-CtxMII resulted in curves that best fit to a one-site model in all striatal regions. Each value represents the means ± S.E.M. of six unlesioned animals. Numbers in parentheses are the 95% confidence intervals (CI).

Region & Peptide	Preferred Model (No. of Sites)	Peptide		Ratio (IC ₅₀ 2/IC ₅₀ 1)	Fraction of Receptors	
		IC ₅₀ 1 (CI)	IC ₅₀ 2 (CI)		f1	f2
		<i>pM</i>			<i>%</i>	
Medial caudate						
α-CtxMII	One		230 (180–310)			100
E11A	Two	0.0097 (0.0028–0.033)	51 (32–80)	5300	34	66
Lateral caudate						
α-CtxMII	One		310 (250–390)			100
E11A	Two	0.0083 (0.0021–0.032)	56 (34–91)	6700	35	65
Ventral putamen						
α-CtxMII	One		260 (220–300)			100
E11A	Two	0.0085 (0.0021–0.033)	38 (27–54)	4400	27	73
Dorsal putamen						
α-CtxMII	One		270 (200–360)			100
E11A	Two	0.0049 (0.0015–0.016)	33 (22–50)	6700	37	63

vestigated the effect of the α -CtxMII analog E11A on ^{125}I - α -CtxMII binding and compared the results with those obtained using unlabeled native α -CtxMII in striatum of monkeys (Fig. 3). This species offers the advantage that $\sim 50\%$ of the nAChRs in striatum are of the $\alpha 6\beta 2^*$ subtype, whereas in mice they comprise only $\sim 15\%$ of the total nAChR population (Kulak et al., 2002b). Consistent with previous results (Quirk et al., 2001), α -CtxMII inhibition of ^{125}I - α -CtxMII in medial caudate yielded a curve that best fit to a one-site model with an $\text{IC}_{50} = \sim 230$ pM (Table 4). In contrast, E11A inhibited ^{125}I - α -CtxMII binding in medial caudate in a biphasic manner (Fig. 3B). Two sites were identified with an IC_{50} of 9.7 fM and 51 pM (that is, a 5300-fold difference in affinity between the two $\alpha 6\beta 2^*$ nAChR populations) (Table 4). In medial caudate, 34% of the sites were of

very high affinity. Similar results were obtained for the other striatal regions, including lateral caudate, ventral putamen, and dorsal putamen (Fig. 3B and Table 4). Specific binding (fmol/mg tissue) in the different striatal areas in unlesioned animals was as follows ($n = 6$): medial caudate, 2.15 ± 0.13 ; lateral caudate, 1.83 ± 0.15 ; ventral putamen, 2.11 ± 0.18 ; and dorsal putamen, 2.03 ± 0.19 . These combined data suggest that E11A discriminates between at least two $\alpha 6\beta 2^*$ nAChR subtypes in unlesioned monkey striatum, whereas α -CtxMII interacts similarly with these two populations.

Preferential Decline of the Very-High-Affinity E11A-Sensitive ^{125}I - α -CtxMII Binding Site in Striatum of Monkeys with Nigrostriatal Damage. Experiments were done to study receptor changes in striatum of monkeys with nigrostriatal damage. The animals were divided into two

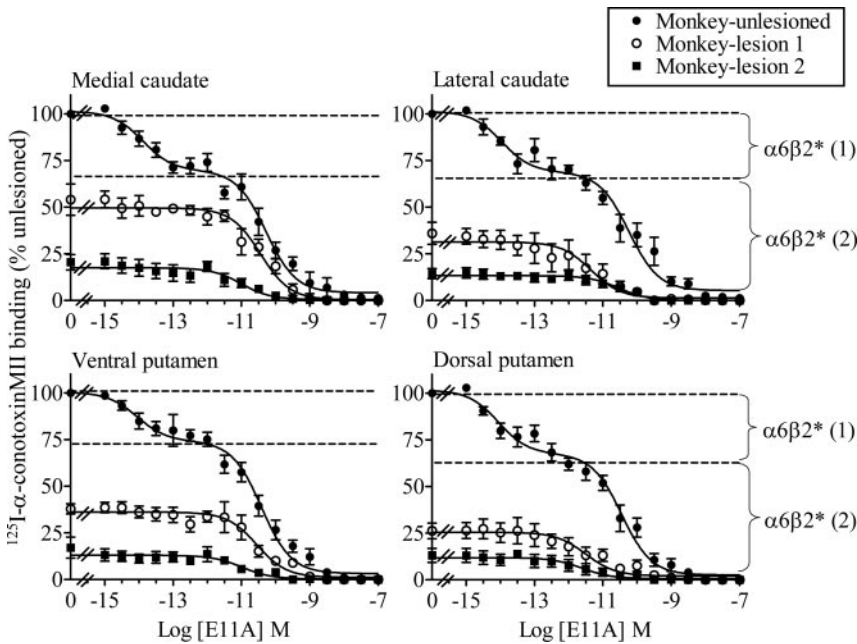


Fig. 4. Nigrostriatal damage results in a selective loss of the very high affinity $\alpha 6\beta 2^*$ nAChR subtype in monkey striatum. Monkeys were injected with saline or MPTP as described under *Materials and Methods*. The lesioned monkeys were divided into two groups based on the extent of loss of striatal ^{125}I - α -CtxMII binding (lesion 1 and lesion 2), with significant decreases in all striatal regions. Competition analysis of E11A inhibition of ^{125}I - α -CtxMII binding in unlesioned striatal regions yielded a biphasic curve binding. In contrast, there was monophasic inhibition of ^{125}I - α -CtxMII binding by E11A (best fit to a one-site model) in striatal regions from MPTP-treated monkeys, suggesting the loss of a subpopulation of $\alpha 6\beta 2^*$ nAChRs with nigrostriatal damage. Similar results were observed in medial and lateral caudate, and ventral and dorsal putamen. Symbols represents mean \pm S.E.M. of four to six monkeys. Where the S.E.M. is not depicted, it fell within the symbol.

TABLE 5

MPTP treatment selectively decreases the high-affinity E11A-sensitive $\alpha 6\beta 2^*$ nAChR population in monkey striatum

^{125}I - α -CtxMII competition curves with varying concentration of E11A were done as described under *Materials and Methods* using striatal sections from unlesioned and MPTP-lesioned monkeys. Biphasic inhibition of ^{125}I - α -CtxMII by E11A (data best fit to a two-site model) suggests the presence of two $\alpha 6\beta 2^*$ nAChR populations in unlesioned monkey striatum. Competition analyses of ^{125}I - α -CtxMII by E11A of the data obtained from either lesioned group yielded monophasic curves, with the loss of the very high affinity component, in all striatal areas. Each value represents the mean \pm S.E.M. of four to six monkeys. Numbers in parentheses are the 95% confidence intervals (CI).

Region & Group	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
					<i>pM</i>	
					<i>%</i>	
Medial caudate						
Monkey-unlesioned	Two	0.0097 (0.0028–0.033)	51 (32–80)	5600	34	66
Monkey-lesion 1	One		38 (24–60)			100
Monkey-lesion 2	One		11 (3.9–30)			100
Lateral caudate						
Monkey-unlesioned	Two	0.0083 (0.0021–0.032)	56 (34–91)	7000	35	65
Monkey-lesion 1	One		5.6 (2.1–15)			100
Monkey-lesion 2	One		31 (13–71)			100
Ventral putamen						
Monkey-unlesioned	Two	0.0085 (0.0021–0.033)	38 (27–54)	4800	27	73
Monkey-lesion 1	One		29 (17–49)			100
Monkey-lesion 2	One		13 (4.4–37)			100
Dorsal putamen						
Monkey-unlesioned	Two	0.0049 (0.0015–0.016)	33 (22–50)	6800	37	63
Monkey-lesion 1	One		3.2 (1.2–8.7)			100
Monkey-lesion 2	One		2.8 (0.78–10)			100

groups based on measurement of the dopamine transporter, which yielded a set of monkeys with less severe declines (monkey-lesion 1) and another with more severe declines (monkey-lesion 2) in ¹²⁵I-RTI-121 binding (Table 2). There were corresponding losses in striatal ¹²⁵I-α-CtxMII binding in the two lesioned monkey groups (Fig. 4), consistent with a previous study (Quik et al., 2001). Striatal ¹²⁵I-α-CtxMII binding in the different striatal regions (Fig. 4) ranged from 36 to 54% of unlesioned animals in the less severely lesioned group and from 12 to 21% of unlesioned animals for the more severely lesioned group. Correlation analyses of ¹²⁵I-RTI-121 to ¹²⁵I-α-CtxMII binding for the different striatal areas yielded values of $r = 0.81$ ($p < 0.001$) for medial caudate, $r = 0.89$ ($p < 0.001$) for lateral caudate, $r = 0.92$ ($p < 0.001$) for ventral putamen, and $r = 0.93$ ($p < 0.001$) for dorsal putamen.

Competition of ¹²⁵I-α-CtxMII binding by E11A in striatum of either lesioned group yielded curves that best fit to a one-site model (Table 5) for all striatal regions (that is, medial caudate, lateral caudate, ventral putamen, and dorsal putamen). The IC₅₀ values of the monophasic curves were more similar to those of the high-affinity sites than the very-high-affinity sites (Table 5). These data suggest that there is selective loss of the very-high-affinity, E11A-sensitive ¹²⁵I-α-CtxMII binding component in striatum of MPTP-treated monkeys, with primarily the high-affinity component remaining after lesioning.

E11A Also Discriminates between Different α6β2* Subtypes in Control Human Striatum. Competition of ¹²⁵I-α-CtxMII binding by α-CtxMII and E11A was initially performed using striatal sections from control human brain (Fig. 5). Unlabeled α-CtxMII completely displaced ¹²⁵I-α-CtxMII binding at 100 nM in both caudate and putamen of control human cases. Inhibition by native α-CtxMII was monophasic (best fit to a single site), as shown previously (Quik et al., 2004), with IC₅₀ values of ~367 and ~392 pM in caudate and putamen, respectively (Fig. 5B). On the other hand, competition of ¹²⁵I-α-CtxMII binding by E11A was biphasic in both the caudate and putamen. There was ≥500-fold difference in IC₅₀ between a very-high-affinity and high-affinity ¹²⁵I-α-CtxMII binding site, with a roughly similar proportion of sites in each population (Fig. 5B, Table 7). These results indicate the presence of at least two E11A-sensitive α6β2* subtypes in human striatum, similar to results in mouse and monkey.

Selective Decrease in the Very-High-Affinity E11A-Sensitive ¹²⁵I-α-CtxMII Binding Site in Parkinson's Disease Striatum. We next performed experiments to evaluate the effect of nigrostriatal damage on the two α6β2* nAChR components in human striatum. The dopamine transporter was first measured to evaluate the extent of nigrostriatal damage. Values for the caudate were 69 ± 11% of control, with somewhat greater declines in putamen of 42 ± 6%, as expected (Table 6). The ¹²⁵I-α-CtxMII binding levels in caudate from control and Parkinson's disease cases was 1.08 ± 0.16 and 0.57 ± 0.04 fmol/mg of tissue, respectively, and in putamen was 0.75 ± 0.10 and 0.30 ± 0.01 fmol/mg of tissue, respectively. The ¹²⁵I-α-CtxMII binding values corresponded to the declines in the dopamine transporter with correlation coefficients of $r = 0.85$ ($p < 0.001$) and $r = 0.93$ ($p < 0.05$) for caudate and putamen, respectively.

To determine whether a select striatal α6β2* subtype was

lost, ¹²⁵I-α-CtxMII inhibition studies were done with E11A (Fig. 6, Table 7). Monophasic curves were obtained in both caudate and putamen from Parkinson's disease cases, with the high-affinity binding site remaining. These data show

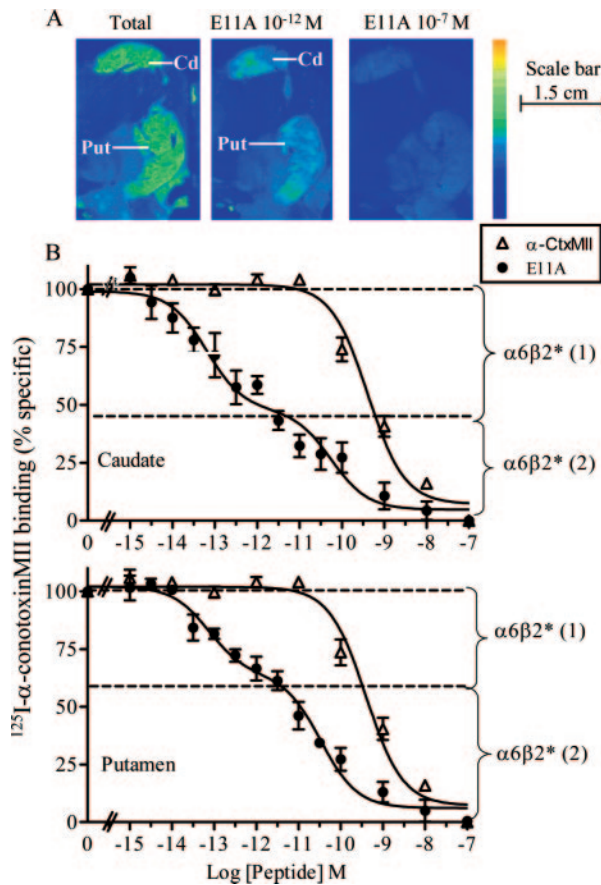


Fig. 5. α-CtxMII E11A, but not native α-CtxMII, distinguishes very-high-affinity and high-affinity α6β2* subtypes in control human striatum. Striatal sections were incubated with 0.5 nM ¹²⁵I-α-CtxMII in the absence and presence of the indicated concentrations of either α-CtxMII or E11A. A, autoradiographic images depicting partial (10⁻¹² M) and complete (10⁻⁷ M) displacement of ¹²⁵I-α-CtxMII binding (Total) by E11A. Background binding was similar to that obtained with 10⁻⁴ M nicotine. B, competition analyses demonstrate the presence of only one α-CtxMII-sensitive ¹²⁵I-α-CtxMII binding site in control caudate and putamen. However, similar to results in mouse and monkey, α-CtxMII E11A identified both very-high-affinity and high-affinity sites (fit best to a two-site model), with >500 difference in IC₅₀ in control human caudate. Similar results were observed in the putamen. The color bar indicates increasing image intensity in the sequence blue, green, yellow, and orange. Symbols represent the mean ± S.E.M. of five control cases and four Parkinson's disease cases. Where the S.E.M. is not depicted, it fell within the symbol. Cd, caudate; Put, putamen.

TABLE 6
Decrease in striatal dopamine transporter in Parkinson's disease striatum
¹²⁵I-RTI-121 binding was done as described under *Materials and Methods*. Each value represents the mean ± S.E.M. of five control cases and four Parkinson's disease cases.

Region	¹²⁵ I-RTI-121	
	Control	Parkinson's Disease
	fmol/mg	
Caudate	4.13 ± 0.42	2.86 ± 0.45*
Putamen	4.36 ± 0.39	1.82 ± 0.29**

Significance of difference from control using a Bonferroni post hoc test: *, $P < 0.05$; **, $P < 0.01$.

that the very-high-affinity, E11A-sensitive ^{125}I - α -CtxMII binding site is lost in striatum of Parkinson's disease cases, similar to results in MPTP-treated mice and monkeys.

Discussion

Results from molecular, pharmacological, and functional studies show that the striatum expresses multiple nAChR subunits, which combine to form distinct ligand-gated ion channels (Wonnacott et al., 2005; Gotti et al., 2006c). These are generally heteromeric and consist of different combinations of α and β subunits, except for $\alpha 7$ nAChRs, which are homomeric. Immunoprecipitation studies with subunit-directed antibodies show that rodent striatum expresses primarily $\alpha 4$, $\alpha 5$, $\alpha 6$, $\alpha 7$, $\beta 2$, and $\beta 3$ subunits (Zoli et al., 2002; Champtiaux et al., 2003). Although the presence of these

multiple subunits has the potential to result in a vast array of pentameric nAChRs, the number of subtypes seems fairly limited; the major populations are $\alpha 6\alpha 4\beta 2\beta 3$, $\alpha 6\beta 2\beta 3$, $\alpha 4\beta 2$, and $\alpha 4\alpha 5\beta 2$ nAChRs (Whiteaker et al., 2002; Zoli et al., 2002; Champtiaux et al., 2003; Salminen et al., 2004; Gotti et al., 2006b). Primate striatum (nonhuman and human) seems to express a slightly different repertoire of nAChR subunits, including $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 6$, $\alpha 7$, $\beta 2$, and $\beta 3$ (Quik et al., 2005; Gotti et al., 2006a,c). Receptor subtypes that have been identified to date include $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ in both human and monkey striatum, as well as $\alpha 4\alpha 2\beta 2$ and $\alpha 3\beta 2^*$ in the monkey.

As mentioned earlier, ^{125}I - α -CtxMII has proved to be a very useful ligand to study $\alpha 6\beta 2^*$ expression and regulation in mice and rats (Quik and McIntosh, 2006). However, because ^{125}I - α -CtxMII also binds to $\alpha 3\beta 2^*$ nAChRs in human and monkey striatum, results are less clear-cut in these latter species (Quik et al., 2005; Gotti et al., 2006a). The use of the α -CtxMII analog E11A therefore seemed appropriate because it discriminates between the $\alpha 3\beta 2^*$ and $\alpha 6\beta 2^*$ subtypes (McIntosh et al., 2004). In oocyte expression studies, 1.0 nM E11A completely inhibited function of chimeric $\alpha 6/\alpha 3\beta 2$ nAChRs, whereas $\alpha 3\beta 2$ receptor-mediated activity was blocked only with ~ 100 nM of the analog (McIntosh et al., 2004). The present results show that 1.0 nM E11A completely inhibited striatal ^{125}I - α -CtxMII binding (0.5 nM). These combined findings suggest that radiolabeled ^{125}I - α -CtxMII binds to $\alpha 6\beta 2^*$ nAChRs in monkey and human striatum. It is possible that differences in binding affinity between these subtypes in striatum are not as great as the functional differences. However, previous work has shown that the IC_{50} for inhibition of ^{125}I - α -CtxMII binding by E11A in rat striatum was similar to a block of striatal dopamine release, as well as to inhibition of nicotinic receptor-mediated responses in oocytes, at least for the high-affinity site, which had been detected in earlier studies (McIntosh et al., 2004; Azam and McIntosh, 2005). Receptor studies in oocytes examining the affinity of E11A for heterologously expressed $\alpha 3\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs may provide a more direct approach to address this issue. However, receptor detection may be difficult in this system because of low receptor expression and/or high nonspecific binding.

Subsequent competition studies using ^{125}I - α -CtxMII unexpectedly showed that E11A discriminated between very-high-affinity (femtomolar) and high-affinity (picomolar) $\alpha 6\beta 2^*$ nAChR population in striatum of mice, as well as

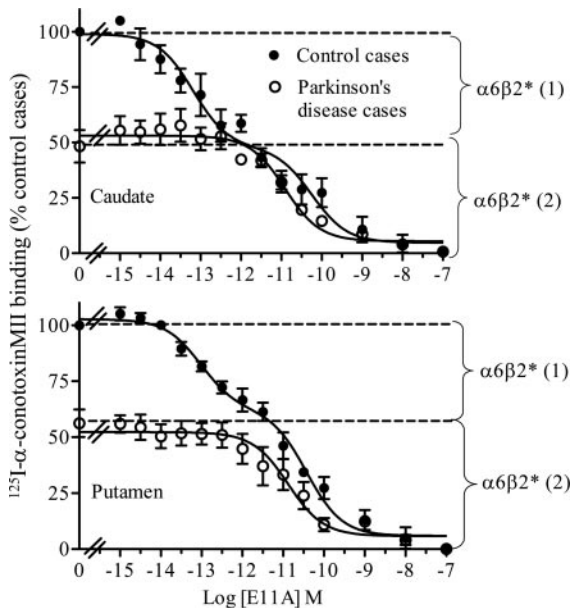


Fig. 6. Loss of the very-high-affinity ^{125}I - α -CtxMII binding component in striatum from Parkinson's disease cases. A decline in ^{125}I - α -CtxMII binding was observed in both caudate and putamen from Parkinson's disease cases. Competition of ^{125}I - α -CtxMII binding by E11A in control human striatum yielded biphasic curves (fit best to a two-site model). However, similar analyses of tissue from Parkinson's disease cases resulted in monophasic curves (fit best to a one-site model), suggesting the loss of the very-high-affinity E11A-sensitive component Parkinson's disease striatum. Symbols represent the mean \pm S.E.M. of five control and four Parkinson's disease cases. Where the S.E.M. is not depicted, it fell within the symbol.

TABLE 7

Selective loss of the very high affinity $\alpha 6\beta 2^*$ nAChR subtype in Parkinson's disease striatum

^{125}I - α -CtxMII competition using varying concentrations of E11A was done as described under *Materials and Methods* using striatal sections from control and Parkinson's disease cases. A biphasic binding curve in control striatum indicated the presence of two $\alpha 6\beta 2^*$ nAChR population. Monophasic inhibition of ^{125}I - α -CtxMII binding by E11A was obtained in Parkinson's disease caudate and putamen, suggesting the loss of the high-affinity subtype. Each value represents the means \pm S.E.M. of five control cases and four Parkinson's disease cases. Numbers in parentheses are the 95% confidence intervals (CI).

Region and Case	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
<i>pM</i>					<i>%</i>	
Caudate						
Control	Two	0.072 (0.026–0.19)	60 (19–190)	860	54	46
Parkinson's Disease	One		11 (6.4–20)			100
Putamen						
Control	Two	0.078 (0.033–0.16)	35 (19–65)	500	41	59
Parkinson's Disease	One		14 (7.1–28)			100

monkeys and humans. Because mice do not express the $\alpha 3$ nAChR subunit, these two binding sites most likely represent two different $\alpha 6\beta 2^*$ subtypes, possibly the previously identified $\alpha 6\alpha 4\beta 2\beta 3$ and $\alpha 6\beta 2\beta 3$ receptors (Zoli et al., 2002; Salmi-nen et al., 2004). This idea is supported by results using $\alpha 4$ knockout mice, in which the $\alpha 6\alpha 4\beta 2\beta 3$ subtype is absent. The missing very-high-affinity E11A-sensitive ^{125}I - α -CtxMII binding site in lesioned mouse striatum may be the $\alpha 6\alpha 4\beta 2\beta 3$ nAChR subtype, with the remaining high-affinity-receptor being the $\alpha 6\beta 2\beta 3$ nAChR population. The similar alterations in the ^{125}I - α -CtxMII competition curves with E11A in monkey and human striatum also suggests a loss of the $\alpha 6\alpha 4\beta 2\beta 3$ nAChR subtype with nigrostriatal damage. One question that arises is whether the presence of the $\alpha 3$ subunit in primate striatum contributes to the different binding affinity sites. Although possible, this seems unlikely, because antibody immunoprecipitation studies show that nAChRs containing the $\alpha 6$ and $\alpha 3$ subunit do not coprecipitate and are thus not part of the same receptor complex (Quik et al., 2005).

The differential interaction of E11A with the $\alpha 6\alpha 4\beta 2\beta 3$ and $\alpha 6\beta 2\beta 3$ subtypes may arise as a result of different molecular configurations of the $\alpha 6$ - $\beta 2$ interfaces in the two receptor complexes. For instance, in the $\alpha 6\beta 2\beta 3$ receptor, there are two $\alpha 6$ - $\beta 2$ interfaces, whereas in the $\alpha 6\alpha 4\beta 2\beta 3$ subtype, the $\alpha 6$ - $\beta 2$ interface is adjacent to an $\alpha 4$ - $\beta 2$ interface that may affect binding of E11A at the $\alpha 6$ - $\beta 2$ recognition site. Another possibility is that the very-high and high-affinity components are variably post-translationally modified (that is, phosphorylated or glycosylated). Such molecular modifications may alter binding such that E11A differentially recognizes the two $\alpha 6\beta 2^*$ subtypes.

The very-high-affinity site varied in IC_{50} value across species with monkey > mouse > human, whereas the high-affinity site followed the rank order mouse > human > monkey. The divergence in IC_{50} values between the two $\alpha 6\beta 2^*$ subtypes in the same species also differed and ranged from several hundred fold in mouse and human striatum, to several thousand fold in monkey striatum. A possible explanation for the diversity in subtype affinities in various species may relate to differential post-translational modifications of the receptor that affect binding of α -CtxMII E11A.

Nigrostriatal damage decreases $\alpha 6\beta 2^*$ nAChRs in experimental animal models and in Parkinson's disease (Quik et al., 2001., 2003, 2004; Bohr et al., 2005). Moreover, the receptor declines are similar although not identical to those of other dopaminergic markers, suggesting that $\alpha 6\beta 2^*$ nAChRs are primarily localized to striatal dopaminergic terminals. It was therefore of interest to determine how the very high and high $\alpha 6\beta 2^*$ components were altered with a nigrostriatal lesion. The very-high-affinity $\alpha 6\beta 2^*$ population was the one primarily decreased with moderate lesioning, whereas the high-affinity component was affected only with a more severe lesion. These data, coupled with the results from the $\alpha 4$ nAChR null mutant mice, suggest that dopaminergic terminals expressing the very high-affinity $\alpha 6\alpha 4\beta 2\beta 3$ nAChR population may be more susceptible to nigrostriatal degeneration. Studies with ^{125}I - α -CtxMII E11A are required to address this possibility; however, radiolabeled toxin is currently not available.

There is precedence in the literature for the concept that select dopaminergic neuron populations are more readily

affected by neurodegenerative insults. Fiber tracts in the substantia nigra differentially project to different striatal areas that appear morphologically distinct (Gerfen et al., 1987b; Langer and Graybiel, 1989). These dopaminergic afferents show a variable susceptibility to nigrostriatal damage. Identification of the molecular markers linked to this differential depletion may provide insight about the mechanisms responsible for the nigrostriatal neurodegeneration. Indeed, it has been suggested that calbindin is positively linked to nigrostriatal dopamine neuron survival (Gerfen et al., 1987a; German et al., 1992; Liang et al., 1996), whereas neuromelanin has been negatively associated (Hirsch et al., 1988; Herrero et al., 1993; Zecca et al., 2003; McCormack et al., 2004), although a definitive relationship of either of these markers with nigrostriatal damage is lacking. In the present study, a clear correlation is observed between the loss of the very-high-affinity E11A-sensitive ^{125}I - α -CtxMII binding sites and nigrostriatal damage. These findings suggest that the very high $\alpha 6\beta 2^*$ population ($\alpha 6\alpha 4\beta 2\beta 3$) may represent a marker for this selectively vulnerable dopaminergic population. Further studies are required to address this possibility.

In summary, the present results are the first to demonstrate the presence of two $\alpha 6\beta 2^*$ nAChRs populations in striatum of mice, monkeys, and humans, which were distinguished using the novel α -CtxMII analog E11A. With respect to their subunit composition, studies with $\alpha 4$ nAChR-null mutant mice suggest that these two populations represent $\alpha 6\alpha 4\beta 2\beta 3$ and $\alpha 6\beta 2\beta 3$ nAChR subtypes. Experiments using striatal tissue from animal models of nigrostriatal damage and from Parkinson's disease cases further indicate that the $\alpha 6\alpha 4\beta 2\beta 3$ is preferentially vulnerable to nigrostriatal damage, thus advancing previous work suggesting that declines reflect a uniform change in striatal $\alpha 6\beta 2^*$ nAChRs. These studies provide an experimental basis for further studies to investigate $\alpha 6\alpha 4\beta 2\beta 3$ nAChR subtype as a marker for nigrostriatal dopaminergic neurons particularly susceptible to nigrostriatal damage. Such work may provide clues concerning the selective nature of the neurodegenerative process in Parkinson's disease.

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