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Multiple CNS nicotinic receptors mediate L-dopa-induced dyskinesias; studies with parkinsonian nicotinic receptor knockout mice^A

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Abstract

Accumulating evidence supports the idea that drugs acting at nicotinic acetylcholine receptors (nAChRs) may be beneficial for Parkinson's disease, a neurodegenerative movement disorder characterized by a loss of nigrostriatal dopaminergic neurons. Nicotine administration to parkinsonian animals protects against nigrostriatal damage. In addition, nicotine and nAChR drugs improve L-dopa-induced dyskinesias, a debilitating side effect of L-dopa therapy which remains the gold-standard treatment for Parkinson's disease. Nicotine exerts its antidyskinetic effect by interacting with multiple nAChRs. One approach to identify the subtypes specifically involved in L-dopa-induced dyskinesias is through the use of nAChR subunit null mutant mice. Previous work with $\alpha 2$ and $\alpha 6$ nAChR knockout mice has shown that $\alpha 6 \alpha 2^*$ nAChRs were necessary for the development/maintenance of L-dopa-induced abnormal involuntary movements (AIMs). The present results in parkinsonian $\alpha 4$ nAChR knockout mice indicate that $\alpha 4 \alpha 2^*$ nAChRs also play an essential role since nicotine did not reduce L-dopa-induced AIMs in such mice. Combined analyses of the data from $\alpha 4$ and $\alpha 6$ knockout mice suggest that the $\alpha 6 \alpha 4 \alpha 2 \alpha 3$ subtype may be critical. In contrast to the studies with $\alpha 4$ and $\alpha 6$ knockout mice, nicotine treatment did reduce L-dopa-induced AIMs in parkinsonian $\alpha 7$ nAChR knockout mice. However, $\alpha 7$ nAChR subunit deletion alone increased baseline AIMs, suggesting that $\alpha 7$ receptors exert an inhibitory influence on L-dopa-induced AIMs. In conclusion, $\alpha 6 \alpha 2^*$, $\alpha 4 \alpha 2^*$ and $\alpha 7$ nAChRs all modulate L-dopa-induced AIMs, although their mode of regulation varies. Thus drugs targeting one or multiple nAChRs may be optimal for reducing L-dopa-induced dyskinesias in Parkinson's disease.

Keywords

Dyskinesias; Nicotine; Nicotinic receptors; Parkinson's disease; Striatum

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Authorship contributions

Participated in research design: MQ, CC, SG; Conducted experiments: CC Provided experimental tools (null mutant and wild type mice): SG; Performed data analyses: MQ, CC; Wrote or contributed to the writing of the manuscript: MQ, CC, SG.

1. Introduction

Parkinson's disease is a progressive neurodegenerative movement disorder characterized by rigidity, tremor and bradykinesia [1–8]. These symptoms arise because of a loss of nigrostriatal dopaminergic neurons. Dopamine precursor therapy with L-dopa alleviates many of the parkinsonian motor symptoms; however, abnormal involuntary movements (AIMs) or dyskinesias arise in most patients with extended treatment. These dyskinesias can range from mild to so severe that they interfere with routine functions of daily living. Presently there are few treatment options for L-dopa-induced dyskinesias. Dopamine agonists are used in early Parkinson's disease as these drugs produce fewer dyskinesias, but they are less effective than L-dopa with disease progression. Amantadine is the only clinically approved pharmacological agent for the treatment of L-dopa-induced dyskinesias; however, its effects are limited and diminish with time [9–12]. Deep brain stimulation has proved very successful for some Parkinson's disease patients; however, this is a serious intervention with all the potential adverse effects associated with brain surgery [13, 14]. Extensive research is therefore underway to identify drugs to reduce dyskinesias by targeting CNS neurotransmitter systems directly or indirectly associated with striatal function [1–8]. This includes the nicotinic acetylcholine receptor (nAChR) system which is anatomically and functionally linked to the dopaminergic system [15].

nAChRs, which mediate the action of acetylcholine, are widely expressed in the striatum as well as other brain regions linked to motor function [15]. These ligand-gated ion channels are composed of different combinations of α and β subunits, with the most common subtypes in striatum being $\alpha 4\beta 2^*$, $\alpha 6\beta 2^*$ and $\alpha 7$ nAChRs. The asterisk indicates the possible presence of other subunits in the receptor complex. The populations most prominently expressed on dopaminergic terminals in the striatum, a primary area that degenerates in Parkinson's disease, are the $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs [15–17]. In addition, $\alpha 4\beta 2^*$ nAChRs are located on striatal GABAergic neurons [18]. Although expressed at a lower density, $\alpha 7$ nAChRs are also present in the striatum on cortical glutamatergic afferents [19]. Thus there is a rationale for considering that all subtypes may influence functions linked to the nigrostriatal system, such as the development of L-dopa-induced dyskinesias.

Consistent with this idea, previous studies have shown that nicotine reduces L-dopa-induced AIMs in parkinsonian rats, mice and monkeys by 50–70% [20–23]. Nicotine treatment attenuated AIMs when administered by injection, drinking water or minipump, with the latter two readily amenable to use in patients either orally or via a patch. Several lines of evidence indicate that nicotine reduces L-dopa-induced dyskinesias by acting at nAChRs. Varenicline, an agonist that interacts with multiple nAChRs [24–30], decreased L-dopa-induced AIMs in 6-hydroxydopamine (6-OHDA)-lesioned rats by ~50%. The nAChR antagonist mecamylamine ameliorated L-dopa-induced AIMs to a similar extent as nicotine. However, its effects on AIMs were not additive with those of nicotine, further suggesting that nicotine acts at nAChRs [22]. Moreover, 5-iodo-A-85380 (A-85380), an agonist that acts selectively at $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs, reduced AIMs supporting the idea that the $\alpha 2^*$ nAChR subtype is important in modulating L-dopa-induced AIMs [31–33].

Further evidence for a role of $\alpha 2^*$ nAChRs stems from experiments with nAChR null mutant mice, which have thus far focused on $\alpha 2$ and $\alpha 6$ nAChR subunit knockout animals. L-dopa-induced AIMs were lower in $\alpha 2$ nAChR subunit knockout mice with a 6-OHDA lesion compared to wild type [34]. Moreover, nicotine treatment did not reduce AIMs in the knockout mice, indicating that $\alpha 2^*$ nAChRs were essential [34]. Similar results were observed with $\alpha 6$ nAChR knockout mice suggesting that $\alpha 6\beta 2^*$ nAChRs are involved in the antidyskinetic effect of nicotine [35].

The present studies were done to investigate whether $\alpha 4 \beta 2^*$ and $\alpha 7$ nAChRs also play a role in L-dopa-induced AIMs. To approach this, we used $\alpha 4 \beta 2^*$ and $\alpha 7$ nAChR null mutant mice. The results show that $\alpha 4 \beta 2^*$ and $\alpha 7$ nAChRs also regulate AIMs, although in a somewhat different fashion from $\alpha 6 \beta 2^*$ nAChRs and from one another. Thus, drugs targeting $\alpha 6 \beta 2^*$, $\alpha 4 \beta 2^*$ and/or $\alpha 7$ nAChRs have the potential to influence the occurrence of L-dopa-induced dyskinesias.

2. Materials and methods

2.1. Animals

Mice with nAChR subunit null mutations ($\alpha 4$, originally obtained from Dr. John Drago, University of Melbourne, Australia; $\alpha 7$, originally obtained from Dr. Arthur Beaudet, Baylor College of Medicine, Houston, USA) were bred onto the C57Bl/6 background for a minimum of 10 generations. They were maintained and produced by heterozygous matings at the Institute for Behavioral Genetics, University of Colorado, Boulder. All care and genotyping procedures were in accordance with the NIH Guide and approved by the Animal Care and Use Committee of the University of Colorado, Boulder. Mice were weaned at 21 days of age and housed 1–5 per cage with same-sex littermates, under a 12 h light/dark cycle with free access to food and water. The mice were genotyped by PCR as previously described [36].

Five wk or more after weaning, the mice were couriered to SRI International, where they were housed 1–5 per cage with same-sex littermates in a temperature- and humidity-controlled environment under a 12 h light/dark cycle with free access to food and water. After an initial acclimation period (2–3 d), the mice were anesthetized with isoflurane (3%) and placed in a Kopf stereotaxic instrument. A burr hole was then drilled through the right side of the skull at the following coordinates relative to Bregma and the dural surface: anteroposterior, -1.2 ; lateral, -1.2 ; ventral, 4.75 . The mice next received a unilateral intracranial injection of $3 \mu\text{g}$ 6-OHDA (Sigma Aldrich, St. Louis, MO, USA) (in $1 \mu\text{l}$) into the medial forebrain bundle to lesion the dopaminergic nigrostriatal pathway, as previously described [35]. Our previous studies show that such a lesion results in ~90% reduction in the striatal dopamine transporter [35]. All procedures were approved by the Institutional Animal Care and Use Committee at SRI International in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Drug treatment

Nicotine (Sigma Aldrich, St. Louis, MO, USA) was administered via the drinking solution containing 2% saccharin (Sigma Aldrich, St. Louis, MO, USA). The mice were first given only saccharin. Two days later nicotine (free base, $25 \mu\text{g/ml}$) was added for 2 days, with the dose increased to $50 \mu\text{g/ml}$ on day 3, to $100 \mu\text{g/ml}$ on day 5, to $200 \mu\text{g/ml}$ on day 8, to a final dose on day 10 of $300 \mu\text{g/ml}$ (nicotine titration phase), as previously described [34, 35, 37–39].

L-dopa methyl ester (L-dopa) and benserazide hydrochloride were both purchased from Sigma Aldrich (St. Louis, MO, USA). L-dopa (3 mg/kg) and benserazide (15 mg/kg) were dissolved in saline and injected sc once daily throughout the study, as previously described [34, 35, 40]. Benserazide is an aromatic amino acid decarboxylase inhibitor given to inhibit the breakdown of L-dopa in the periphery.

2.3. Behavioral testing

L-dopa-induced AIMs were rated as described previously [34, 35, 40]. Individual mice were placed in a cylinder and rated every 15 min for 1 min over a 2 h period. Each AIM subtype

was scored on a frequency scale from 0 to 4 (0 = no dyskinesia; 1 = occasional dyskinesia displayed for < 50% of the observation time; 2 = sustained dyskinesia displayed for > 50% of the observation time; 3 = continuous dyskinesia; 4 = continuous dyskinesia not interruptible by external stimuli). The animals were also rated on an amplitude scale, consisting of two levels. Level A indicated oral AIMs without tongue protrusion, forelimb AIMs without the shoulder engaged or axial AIMs with body twisting < 60°. Level B indicated oral AIMs with tongue protrusion, forelimb AIMs with the shoulder engaged or axial AIMs with body twisting > 60°. The integrated scores for frequency and amplitude of AIMs used for data analysis were calculated as 1A = 1, 1B = 2, 2A = 2, 2B = 4, 3A = 4, 3B = 6, 4A = 6, 4B = 8. This allowed for scores for any one component (oral, forelimb or axial) to range from 0 to 8, with a maximum possible total score per time point of 24. All rating was done in a blinded fashion.

To evaluate parkinsonism we used the forelimb asymmetry test [34, 35, 41, 42]. Forelimb use was assessed in a plexiglass cage for a 3 min period, with mirrors strategically placed to observe paw movement. Wall exploration was expressed in terms of the % use of the impaired forelimb (contralateral) compared to the total number of limb use movements. All rating was done in a blinded fashion.

2.4. Plasma cotinine levels

Blood (~0.2 ml) was taken from the ocular vein under isoflurane anesthesia and plasma prepared. Cotinine levels were determined throughout the study using an EIA kit (Orasure Technologies, Bethlehem, PA).

2.5. Data analyses

GraphPad Prism (San Diego, CA) was used for statistical analyses. Significance was determined using a Mann-Whitney test or two-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test. A level of 0.05 was considered significant. Values are the mean $\pm$ SEM of the indicated number of mice.

3. Results

3.1. Studies with $\alpha 4$ nAChR(–/–) and wildtype mice

3.1.1. No change in parkinsonism in $\alpha 4$ (–/–) nAChR mice compared to wild type— 4 nAChR knockout and wild type mice were lesioned and treated with nicotine and L-dopa as depicted in the timeline to Fig. 1. Experiments were first done to test whether the absence of $\alpha 4$ nAChRs affected parkinsonism after a unilateral 6-OHDA lesion. No difference was observed in the forelimb asymmetry test between wild type and $\alpha 4$ nAChR subunit knockout mice (Table 1).

3.1.2. No change in baseline L-dopa-induced AIM scores in saccharin-treated $\alpha 4$ (–/–) nAChR compared to wild type mice— Wild type and $\alpha 4$ knockout mice were subsequently treated with L-dopa, which resulted in the development of stable AIMs after 2 wk [34, 35]. Our previous data had shown that total L-dopa-induced AIMs were variable in mice and that the scores tended to fall into two categories, moderate (< 35) and high AIM scores (> 35) [34, 35]. Our earlier results also showed that the effects of genetic deletions and drugs may vary with AIM severity [34, 35, 43]. The mice in the current study were therefore divided into 2 groups, those with moderate and those with high AIMs, and the data presented in this fashion in all figures and tables. The results in Table 2 show that there were no differences in L-dopa-induced AIM scores in saccharin-treated wild type compared to saccharin-treated $\alpha 4$ nAChR knockout mice in either the moderate or high AIMs group. Thus, the $\alpha 4$ genotype did not affect baseline L-dopa-induced AIM levels.

3.1.3. Nicotine treatment reduces L-dopa-induced AIMs in wild type but not $\alpha 4(-/-)$ nAChR mice—The effect of nicotine treatment was next assessed on L-dopa-induced AIM scores in 4 wild type and knockout mice, with the data for the mice with high AIM scores shown in Fig. 2. There was a decrease in AIM scores in the nicotine-treated 4 wild type mice, with a significant main effect for total AIMs ($F(1,42) = 6.53, p < 0.05$), oral AIMs ($F(1,42) = 5.99, p < 0.05$) and forelimb AIMs ($F(1,42) = 5.67, p < 0.05$) by two-way ANOVA. However, there were no significant main effects of treatment in the 4 nAChR knockout mice. Similar results were obtained in mice with moderate AIM scores, that is, nicotine treatment reduced L-dopa-induced AIMs in the wild type but not 4 nAChR subunit null mutant mice. In the moderately dyskinetic 4 wild type mice, there was a significant main effect of nicotine treatment on total AIMs ($F(1,96) = 6.83, p < 0.05$), oral AIMs ($F(1,96) = 8.21, p < 0.01$) and forelimb AIMs ($F(1,96) = 8.02, p < 0.01$) by two-way ANOVA. By contrast, there was only a significant main effect of nicotine treatment on oral AIMs in the 4 knockout mice ($F(1,60) = 5.85, p < 0.05$). These data, combined with our previous investigations [34, 35] support a role for 4 2* nAChRs in the nicotine-mediated reduction in L-dopa-induced AIMs.

3.1.4. Plasma cotinine levels in $\alpha 4(-/-)$ nAChR and wild type mice—Plasma cotinine levels were measured as an index of nicotine consumption. Cotinine, a primary nicotine metabolite, provides a relatively good index of nicotine intake because of its long half-life (18 h) [37, 44]. Plasma cotinine levels were similar in nicotine-treated wild type and 4 knockout mice 3 months after the start of nicotine treatment (Table 3) and remained at comparable levels throughout the study.

3.2. Studies with $\alpha 7$ nAChR($-/-$) and wildtype mice

3.2.1. No change in parkinsonism in $\alpha 7$ nACh ($-/-$) mice compared to wild type—7 nAChR knockout and wild type mice were lesioned and treated with nicotine and L-dopa as depicted in the timeline to Fig. 3. Experiments were performed to test whether the absence of 7* nAChRs affected parkinsonism after a unilateral 6-OHDA lesion. No differences were observed in the forelimb asymmetry test between wild type and 7 knockout mice (Table 1).

3.2.2. Increase in baseline L-dopa-induced AIMs in saccharin-treated $\alpha 7(-/-)$ nAChR compared to wild type mice—Wild type and 7 nAChR knockout mice were lesioned and treated with L-dopa as depicted in the timeline in Fig. 3. As described earlier for the 4($-/-$) nAChR studies, the mice were divided into two subgroups, those with moderate and those with high AIM scores. The results in Fig. 4 show that there was increased expression of L-dopa-induced AIMs in the saccharin-treated 7 knockout mice compared to the wild type littermates in both the moderate and high AIMs group. In the moderate AIMs group, there was a significant main effect of genotype on total ($F(1,88) = 15.52, p < 0.001$), oral ($F(1,88) = 18.91, p < 0.001$) and forelimb ($F(1,88) = 11.43, p < 0.01$) AIMs. In the high AIMs group, there was also a significant main effect of genotype for total ($F(1,165) = 16.90, p < 0.001$), oral ($F(1,165) = 11.63, p < 0.001$), axial ($F(1,165) = 10.88, p < 0.01$) and forelimb ($F(1,165) = 16.66, p < 0.001$) AIMs. In the mice with moderate AIMs, the enhanced AIM levels in the 7 knockout mice were more pronounced with the first wk of saccharin treatment and declined towards the end of the study period (23 wk). In the high AIMs group, the scores were generally elevated for the entire treatment period.

3.2.3. Nicotine treatment reduces L-dopa-induced AIMs in both wild type and $\alpha 7(-/-)$ nAChR mice—We next tested the effect of nicotine treatment in 7 wild type and knockout mice. After 5 wk of treatment, there was no effect of nicotine on L-dopa-induced AIMs in wild type mice (Fig. 5). These data are consistent with our previous studies

which show that several months of treatment is required to reduce AIMs in L-dopa-primed mice [34]. However, after 5 wk, nicotine did reduce AIMs in $\gamma 7$ nAChR knockout mice, which had elevated AIM levels. Similar results were observed at this time point with mice that exhibited either moderate or high AIMs. After 14 wk of treatment, nicotine reduced L-dopa-induced AIMs in both the moderate and high AIM scoring groups of the $\gamma 7(-/-)$ but only in the high AIMs group of the wild type mice (Fig. 6). By 23 wk of treatment, nicotine reduced L-dopa-induced AIMs to a similar extent in $\gamma 7(-/-)$ mice and wild type mice (Fig. 7).

3.2.4. Plasma cotinine levels in $\alpha 7$ nAChR($-/-$) and wild type mice—Plasma cotinine levels were measured to ensure that the mice were receiving adequate levels of nicotine in the drinking water. Plasma cotinine levels were comparable in nicotine-treated wild type and $\gamma 7$ knockout mice 2 months after the start of nicotine treatment (Table 3), with similar plasma levels throughout the study.

4. Discussion

The use of nAChR null mutant mice has proved a valuable resource for identifying the role of nAChRs in CNS functions linked to the dopaminergic system, including addiction and locomotor activity [45–48]. Here, we used $\alpha 4$ and $\gamma 7$ nAChR subunit knockout mice to understand if these subtypes are involved in L-dopa-induced AIMs. The results show that the $\alpha 4^*$ nAChR appears to play an important role since nicotine generally failed to decrease L-dopa-induced AIMs in $\alpha 4(-/-)$ nAChR subunit mice. Nicotine treatment did reduce AIMs in $\gamma 7$ nAChR knockout mice; however, such mice exhibited higher L-dopa-induced AIM levels suggesting that $\gamma 7$ nAChRs exert an inhibitory influence. Thus both $\alpha 4^*$ and $\gamma 7$ nAChRs appear to regulate L-dopa-induced AIMs, possibly in an opposing fashion.

Previous findings had also indicated that $\alpha 6 \beta 2^*$ nAChRs are involved in the nicotine-mediated decline in L-dopa-induced AIMs. Both $\alpha 2$ and $\alpha 6$ nAChR subunit deletion decreased baseline AIMs, as well as the ability of nicotine to further reduce L-dopa-induced AIMs [34, 35] (summarized in Table 4). Thus both $\alpha 4 \beta 2^*$ and $\alpha 6 \beta 2^*$ nAChR subtypes appear involved in the nicotine-mediated decrease in L-dopa-induced AIMs, although in a somewhat different manner. One possibility is that the $\alpha 6 \beta 4 \beta 2 \beta 3$ nAChR subtype is critical for the effect of nicotine on AIMs. Our rationale for this suggestion is based on findings showing that nicotine does not decrease AIMs in $\alpha 6$ null mutants that lack the $\alpha 6 \beta 4 \beta 2 \beta 3$ and $\alpha 6 \beta 2$ subtypes, but still express $\alpha 4 \beta 2$ and $\alpha 4 \beta 5 \beta 2$ nAChR [34, 35]. Moreover, nicotine did not reduce AIMs in $\alpha 4$ null mutants that lack $\alpha 6 \beta 4 \beta 2 \beta 3$, $\alpha 4 \beta 2$ and $\alpha 4 \beta 5 \beta 2$ nAChRs. The population common to both studies is the $\alpha 6 \beta 4 \beta 2 \beta 3$ receptor. The idea that this latter subtype is important is consistent with reports showing that $\alpha 6 \beta 4 \beta 2 \beta 3$ nAChRs mediate nicotine's effects on dopamine neuron firing and endogenous dopamine release [49–51].

The suggestion that compounds interacting with $\alpha 2^*$ nAChRs may reduce L-dopa-induced dyskinesias agrees with previous studies using nAChR drugs. A-85380 and a series of Targacept compounds that selectively act at $\alpha 2^*$ nAChRs all reduced AIMs in 6-OHDA-lesioned rats [31, 52]. There are currently no $\alpha 4 \beta 2^*$ nor $\alpha 6 \beta 2^*$ selective nAChR compounds available to evaluate the separate role of these subtypes in behavior [30, 52, 53]. To date, the effect of drugs targeting $\gamma 7$ nAChRs has not yet been examined.

Our data show that deletion of $\gamma 7$ nAChRs also affects L-dopa-induced AIMs, albeit in a manner distinct from that of $\alpha 4^*$ or $\alpha 6^*$ nAChRs. An increase in baseline L-dopa-induced AIMs was observed in $\gamma 7$ null mutant mice, suggesting that this subtype has an inhibitory impact on the development of AIMs. However, nicotine still reduced L-dopa-induced AIMs in $\gamma 7$ knockout mice. This improvement in AIMs in the $\gamma 7$ nAChR knockout mice is

probably mediated via $\alpha 4 \beta 2^*$ and/or $\alpha 6 \beta 2^*$ nAChRs, which are unaffected by deletion of the $\alpha 7$ nAChR transcript [54].

A point of note is that the regulation of L-dopa-induced AIMs by $\alpha 2^*$ nAChRs appears quite distinct from that by $\alpha 7$ nAChRs. Although the $\alpha 6$ deletion reduced baseline L-dopa-induced AIM scores, both $\alpha 6$ and $\alpha 4$ nAChR subunit deletion led to a loss of the nicotine-mediated antidyskinetic effect (Table 4). By contrast, $\alpha 7$ nAChR subunit deletion resulted in an increase in baseline L-dopa-induced AIMs activity, but did not affect the antidyskinetic effect of nicotine. These differences may relate to the fact that $\alpha 7$ and the two $\alpha 2^*$ nAChR subtypes are functionally quite distinct. For instance, $\alpha 7$ nAChRs are much more permeable to calcium, desensitize more rapidly and are linked to different intracellular cell signaling steps compared to $\alpha 2^*$ nAChRs [55–59]. These unique molecular properties may provide a basis for the varying effects of $\alpha 7$ and $\alpha 2^*$ nAChR-directed drugs on behaviors, such as L-dopa-induced dyskinesias.

Although $\alpha 6 \beta 2^*$, $\alpha 4 \beta 2^*$ and $\alpha 7$ nAChRs are all present in the striatum, it is not known whether alterations in baseline L-dopa-induced AIMs and/or the lack of effectiveness of nicotine in the $\alpha 4$, $\alpha 6$ or $\alpha 7$ nAChR null mutant mice are due to deletion of receptors in the striatum or in other brain areas. For instance, $\alpha 4 \beta 2^*$ and $\alpha 7$ nAChRs are widely distributed throughout the CNS. This includes numerous other areas linked to motor control and coordination, such as the thalamus, cortex and cerebellum. Any one, or a combination, of these regions may play a role in the occurrence of L-dopa-induced AIMs. The $\alpha 6 \beta 2^*$ nAChRs are somewhat more selectively localized to catecholaminergic regions in the brain. However, although they are most prominently present in the striatum, $\alpha 6 \beta 2^*$ nAChRs are also present in the substantia nigra, the locus coeruleus and its projection areas, as well as multiple components of the visual system [60–62]. Selective localized ablation of nAChRs is necessary to identify the populations involved in the generation of L-dopa-induced AIMs.

In conclusion, the present results using nAChR knockout mice suggest that $\alpha 6 \beta 2^*$, $\alpha 4 \beta 2^*$ and $\alpha 7$ nAChRs all influence the expression of L-dopa-induced AIMs in varying ways. Thus, select nAChR drugs interacting at one or more nAChR subtypes may prove most useful as antidyskinetic agents.

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Glossary

α -CtxMII	α -conotoxinMII
nAChR	nicotinic acetylcholine receptor
	*indicates the possible presence of other subunits in the receptor complex

References

- Poewe W, Mahlknecht P, Jankovic J. Emerging therapies for Parkinson's disease. *Curr Opin Neurol.* 2012; 25:448–459. [PubMed: 22772874]
- Brotchie J, Jenner P. New approaches to therapy. *Int Rev Neurobiol.* 2011; 98:123–150. [PubMed: 21907085]
- Carta M, Bezard E. Contribution of pre-synaptic mechanisms to l-DOPA-induced dyskinesia. *Neuroscience.* 2011; 198:245–251. [PubMed: 21840375]
- Fisone G, Bezard E. Molecular mechanisms of l-DOPA-induced dyskinesia. *Int Rev Neurobiol.* 2011; 98:95–122. [PubMed: 21907084]

5. Iravani MM, Jenner P. Mechanisms underlying the onset and expression of levodopa-induced dyskinesia and their pharmacological manipulation. *J Neural Transm.* 2011; 118:1661–1690. [PubMed: 21881839]
6. Cenci MA, Konradi C. Maladaptive striatal plasticity in L-DOPA-induced dyskinesia. *Progress in brain research.* 2010; 183C:209–233. [PubMed: 20696322]
7. Prashanth LK, Fox S, Meissner WG. L-Dopa-induced dyskinesia-clinical presentation, genetics, and treatment. *Int Rev Neurobiol.* 2011; 98:31–54. [PubMed: 21907082]
8. Rascol O, Lozano A, Stern M, Poewe W. Milestones in Parkinson's disease therapeutics. *Mov Disord.* 2011; 26:1072–1082. [PubMed: 21626552]
9. Gottwald MD, Aminoff MJ. Therapies for dopaminergic-induced dyskinesias in parkinson disease. *Annals of neurology.* 2011; 69:919–927. [PubMed: 21681795]
10. Sawada H, Oeda T, Kuno S, Nomoto M, Yamamoto K, Yamamoto M, et al. Amantadine for dyskinesias in Parkinson's disease: a randomized controlled trial. *PLoS One.* 2010; 5:e15298. [PubMed: 21217832]
11. Huot P, Johnston TH, Koprich JB, Fox SH, Brotchie JM. The pharmacology of L-DOPA-induced dyskinesia in Parkinson's disease. *Pharmacol Rev.* 2013; 65:171–222. [PubMed: 23319549]
12. Hinson VK. Parkinson's disease and motor fluctuations. *Curr Treat Options Neurol.* 2010; 12:186–199. [PubMed: 20842581]
13. Sankar T, Lozano AM. Surgical approach to L-dopa-induced dyskinesias. *Int Rev Neurobiol.* 2011; 98:151–171. [PubMed: 21907086]
14. Fasano A, Daniele A, Albanese A. Treatment of motor and non-motor features of Parkinson's disease with deep brain stimulation. *Lancet Neurol.* 2012; 11:429–442. [PubMed: 22516078]
15. Quik M, Wonnacott S. $\alpha_6\beta_2^*$ and $\alpha_4\beta_2^*$ Nicotinic Acetylcholine Receptors As Drug Targets for Parkinson's Disease. *Pharmacol Rev.* 2011; 63:938–966. [PubMed: 21969327]
16. Grady SR, Salminen O, Lavery DC, Whiteaker P, McIntosh JM, Collins AC, et al. The subtypes of nicotinic acetylcholine receptors on dopaminergic terminals of mouse striatum. *Biochem Pharmacol.* 2007; 74:1235–1246. [PubMed: 17825262]
17. Millar NS, Gotti C. Diversity of vertebrate nicotinic acetylcholine receptors. *Neuropharmacology.* 2009; 56:237–246. [PubMed: 18723036]
18. Luo R, Janssen MJ, Partridge JG, Vicini S. Direct and GABA-mediated indirect effects of nicotinic ACh receptor agonists on striatal neurones. *J Physiol.* 2013; 591:203–217. [PubMed: 23045343]
19. Kaiser S, Wonnacott S. α -bungarotoxin-sensitive nicotinic receptors indirectly modulate $[(3)H]$ dopamine release in rat striatal slices via glutamate release. *Mol Pharmacol.* 2000; 58:312–318. [PubMed: 10908298]
20. Bordia T, Campos C, Huang L, Quik M. Continuous and intermittent nicotine treatment reduces L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesias in a rat model of Parkinson's disease. *J Pharmacol Exp Ther.* 2008; 327:239–247. [PubMed: 18650244]
21. Quik M, Cox H, Parameswaran N, O'Leary K, Langston JW, Di Monte D. Nicotine reduces levodopa-induced dyskinesias in lesioned monkeys. *Annals of neurology.* 2007; 62:588–596. [PubMed: 17960553]
22. Bordia T, Campos C, McIntosh JM, Quik M. Nicotinic receptor-mediated reduction in L-dopa-induced dyskinesias may occur via desensitization. *J Pharmacol Exp Ther.* 2010; 333:929–938. [PubMed: 20200117]
23. Quik M, Mallela A, Chin M, McIntosh JM, Perez XA, Bordia T. Nicotine-mediated improvement in L-dopa-induced dyskinesias in MPTP-lesioned monkeys is dependent on dopamine nerve terminal function. *Neurobiol Dis.* 2013; 50:30–41. [PubMed: 23009753]
24. Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, et al. Varenicline: an $\alpha_4\beta_2$ nicotinic receptor partial agonist for smoking cessation. *J Med Chem.* 2005; 48:3474–3477. [PubMed: 15887955]
25. Rollema H, Coe JW, Chambers LK, et al. Rationale, pharmacology and clinical efficacy of partial agonists of $\alpha_4(4)\beta_2(2)$ nACh receptors for smoking cessation. *Trends Pharmacol Sci.* 2007; 28:316–325. [PubMed: 17573127]

26. Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, et al. Pharmacological profile of the $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. *Neuropharmacology*. 2007; 52:985–994. [PubMed: 17157884]
27. Gonzales D, Rennard SI, Nides M, Oncken C, Azoulay S, Billing CB, et al. Varenicline, an $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist, vs sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. *Jama*. 2006; 296:47–55. [PubMed: 16820546]
28. Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, et al. Efficacy of varenicline, an $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. *Jama*. 2006; 296:56–63. [PubMed: 16820547]
29. Mihalak KB, Carroll FI, Luetje CW. Varenicline is a partial agonist at $\alpha 4\beta 2$ and a full agonist at $\alpha 7$ neuronal nicotinic receptors. *Mol Pharmacol*. 2006; 70:801–805. [PubMed: 16766716]
30. Bordia T, Hrachova M, Chin M, McIntosh JM, Quik M. Varenicline is a potent partial agonist at $\alpha 6\beta 2^*$ nicotinic acetylcholine receptors in rat and monkey striatum. *J Pharmacol Exp Ther*. 2012; 342:327–34. [PubMed: 22550286]
31. Huang LZ, Campos C, Ly J, Carroll FI, Quik M. Nicotinic receptor agonists decrease L-dopa-induced dyskinesias most effectively in moderately lesioned parkinsonian rats. *Neuropharmacology*. 2011; 60:861–868. [PubMed: 21232546]
32. Mukhin AG, Gundisch D, Horti AG, Koren AO, Tamagnan G, Kimes AS, et al. 5-Iodo-A-85380, an $\alpha 4\beta 2$ subtype-selective ligand for nicotinic acetylcholine receptors. *Mol Pharmacol*. 2000; 57:642–649. [PubMed: 10692507]
33. Kulak JM, Sum J, Musachio JL, McIntosh JM, Quik M. 5-Iodo-A-85380 binds to α -conotoxin MII-sensitive nicotinic acetylcholine receptors (nAChRs) as well as $\alpha 4\beta 2^*$ subtypes. *J Neurochem*. 2002; 81:403–406. [PubMed: 12064487]
34. Huang L, Grady SR, Quik M. Nicotine Reduces L-Dopa-Induced Dyskinesias by Acting at $\beta 2$ Nicotinic Receptors. *J Pharmacol Exp Ther*. 2011; 338:932–941. [PubMed: 21665941]
35. Quik M, Park KM, Hrachova M, Mallela A, Huang LZ, McIntosh JM, et al. Role for $\alpha 6$ nicotinic receptors in l-dopa-induced dyskinesias in parkinsonian mice. *Neuropharmacology*. 2012; 63:450–459. [PubMed: 22579614]
36. Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC, et al. Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Mol Pharmacol*. 2004; 65:1526–1535. [PubMed: 15155845]
37. Lai A, Parameswaran N, Khwaja M, Whiteaker P, Lindstrom JM, Fan H, et al. Long-term nicotine treatment decreases striatal $\alpha 6^*$ nicotinic acetylcholine receptor sites and function in mice. *Mol Pharmacol*. 2005; 67:1639–1647. [PubMed: 15681595]
38. Pietila K, Ahtee L. Chronic Nicotine Administration in the Drinking Water Affects the Striatal Dopamine in Mice. *Pharmacol Biochem Behav*. 2000; 66:95–103. [PubMed: 10837848]
39. Sparks JA, Pauly JR. Effects of continuous oral nicotine administration on brain nicotinic receptors and responsiveness to nicotine in C57Bl/6 mice. *Psychopharmacology (Berl)*. 1999; 141:145–153. [PubMed: 9952038]
40. Cenci MA, Lundblad M. Ratings of L-DOPA-induced dyskinesia in the unilateral 6-OHDA lesion model of Parkinson's disease in rats and mice. *Curr Protoc Neurosci*. 2007:1–23. Chapter 9. [PubMed: 18428664]
41. Schallert T, Fleming SM, Leasure JL, Tillerson JL, Bland ST. CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. *Neuropharmacology*. 2000; 39:777–787. [PubMed: 10699444]
42. Brooks SP, Dunnett SB. Tests to assess motor phenotype in mice: a user's guide. *Nat Rev Neurosci*. 2009; 10:519–529. [PubMed: 19513088]
43. Carta M, Carlsson T, Kirik D, Bjorklund A. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. *Brain*. 2007; 130:1819–1833. [PubMed: 17452372]

44. Matta SG, Balfour DJ, Benowitz NL, Boyd RT, Buccafusco JJ, Caggiula AR, et al. Guidelines on nicotine dose selection for in vivo research. *Psychopharmacology (Berl)*. 2007; 190:269–319. [PubMed: 16896961]
45. Fowler CD, Arends MA, Kenny PJ. Subtypes of nicotinic acetylcholine receptors in nicotine reward, dependence, and withdrawal: evidence from genetically modified mice. *Behav Pharmacol*. 2008; 19:461–84. [PubMed: 18690103]
46. Cordero-Erausquin M, Marubio LM, Klink R, Changeux JP. Nicotinic receptor function: new perspectives from knockout mice. *Trends Pharmacol Sci*. 2000; 21:211–217. [PubMed: 10838608]
47. Champiaux N, Changeux JP. Knockout and knockin mice to investigate the role of nicotinic receptors in the central nervous system. *Prog Brain Res*. 2004; 145:235–251. [PubMed: 14650919]
48. Changeux JP. Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. *Nat Rev Neurosci*. 2010; 11:389–401. [PubMed: 20485364]
49. Exley R, Maubourguet N, David V, Eddine R, Evrard A, Pons S, et al. Distinct contributions of nicotinic acetylcholine receptor subunit alpha4 and subunit alpha6 to the reinforcing effects of nicotine. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:7577–7582. [PubMed: 21502501]
50. Liu L, Zhao-Shea R, McIntosh JM, Gardner PD, Tapper AR. Nicotine persistently activates ventral tegmental area dopaminergic neurons via nicotinic acetylcholine receptors containing alpha4 and alpha6 subunits. *Mol Pharmacol*. 2012; 81:541–548. [PubMed: 22222765]
51. Zhao-Shea R, Liu L, Soll LG, Improgo MR, Meyers EE, McIntosh JM, et al. Nicotine-mediated activation of dopaminergic neurons in distinct regions of the ventral tegmental area. *Neuropsychopharmacology*. 2011; 36:1021–1032. [PubMed: 21289604]
52. Quik M, Campos C, Bordia T, Strachan JP, Zhang J, McIntosh JM, et al. alpha4beta2 nicotinic receptors play a role in the nAChR-mediated decline in l-dopa-induced dyskinesias in parkinsonian rats. *Neuropharmacology*. 2013; 71:191–203. [PubMed: 23583932]
53. Marks MJ, Wageman CR, Grady SR, Gopalakrishnan M, Briggs CA. Selectivity of ABT-089 for alpha4beta2* and alpha6beta2* nicotinic acetylcholine receptors in brain. *Biochem Pharmacol*. 2009; 78:795–802. [PubMed: 19481067]
54. Seguela P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW. Molecular cloning, functional properties, and distribution of rat brain alpha 7: a nicotinic cation channel highly permeable to calcium. *J Neurosci*. 1993; 13:596–604. [PubMed: 7678857]
55. Giniatullin R, Nistri A, Yakel JL. Desensitization of nicotinic ACh receptors: shaping cholinergic signaling. *Trends Neurosci*. 2005; 28:371–378. [PubMed: 15979501]
56. Picciotto MR, Addy NA, Mineur YS, Brunzell DH. It is not "either/or": Activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. *Prog Neurobiol*. 2008; 84:329–342. [PubMed: 18242816]
57. Wonnacott S, Sidhpura N, Balfour DJ. Nicotine: from molecular mechanisms to behaviour. *Curr Opin Pharmacol*. 2005; 5:53–59. [PubMed: 15661626]
58. Changeux JP. Allosteric receptors: from electric organ to cognition. *Annual review of pharmacology and toxicology*. 2010; 50:1–38.
59. Quik M, Perez XA, Bordia T. Nicotine as a potential neuroprotective agent for Parkinson's disease. *Mov Disord*. 2012; 27:947–957. [PubMed: 22693036]
60. Whiteaker P, McIntosh JM, Luo S, Collins AC, Marks MJ. 125I-alpha-conotoxin MII identifies a novel nicotinic acetylcholine receptor population in mouse brain. *Mol Pharmacol*. 2000; 57:913–925. [PubMed: 10779374]
61. Quik M, Polonskaya Y, Kulak JM, McIntosh JM. Vulnerability of 125I-alpha-conotoxin MII binding sites to nigrostriatal damage in monkey. *J Neurosci*. 2001; 21:5494–5500. [PubMed: 11466420]
62. Champiaux N, Han ZY, Bessis A, Rossi FM, Zoli M, Marubio L, et al. Distribution and pharmacology of alpha 6-containing nicotinic acetylcholine receptors analyzed with mutant mice. *J Neurosci*. 2002; 22:1208–1217. [PubMed: 11850448]

Fig. 1.

Treatment timeline for 4 nAChR null mutant and wild type mice. All mice were unilaterally lesioned with 6-OHDA. At wk 2, parkinsonism was assessed using the forelimb asymmetry test. One wk later, the mice were injected with L-dopa (3 mg/kg) plus benserazide (15 mg/kg) for the duration of the study. After L-dopa-induced AIMs had stably developed, the mice were given nicotine in the drinking water with the dose gradually increased to 300 μ g/ml (nicotine titration phase). The numbers in the white squares represent the wk on nicotine treatment.

Fig. 2.

Nicotine treatment reduces L-dopa-induced AIMS in wild type but not $\alpha 4(-/-)$ nAChR mice. Lesioned mice were injected once daily with L-dopa (3 mg/kg) plus benserazide (15 mg/kg) and provided with nicotine in the drinking water. Mice were evaluated for oral, axial, forelimb and total AIMS for 1 min every 15 min over a 2-h period. The times on the x-axis correspond to the wk on nicotine treatment (white boxes in Fig. 1). Values represent the mean $\pm$ SEM of 4–5 animals. Where the SEM is not shown, it fell within the symbol. # indicates a significant ($p < 0.05$) main effect of nicotine using two-way ANOVA.

Fig. 3.

Treatment timeline for 7 nAChR null mutant and wild type mice. All mice were unilaterally lesioned with 6-OHDA. Parkinsonism was evaluated 2 wk later using the forelimb asymmetry test. Daily L-dopa (3 mg/kg) plus benserazide (15 mg/kg) dosing was initiated 3 wk after lesioning and continued throughout the study. After L-dopa-induced AIMs had stably developed, nicotine was given in the drinking water, as indicated in the timeline. The numbers in the white squares represent the wk on nicotine treatment. L-dopa-induced AIMs were measured throughout the study.

Fig. 4.

Increase in L-dopa-induced AIMs in saccharin-treated $\alpha 7(-/-)$ nAChR compared to wild type mice. 6-OHDA-lesioned mice were injected once daily with L-dopa (3 mg/kg) plus benserazide (15 mg/kg). They were evaluated for oral, axial, forelimb and total AIMs for 1 min every 15 min over a 2-h period. The mice were subdivided into two groups, those with moderate and those with high AIM scores. The times on the x-axis correspond to the wk on nicotine treatment (white boxes in Fig. 3). Values represent the mean $\pm$ SEM of 4–6 mice in the moderate AIMs group and 8–9 mice in the high AIMs group. Where the SEM is not shown, it fell within the symbol. There was a significant main effect of genotype using two-way ANOVA (## $p < 0.01$, ### $p < 0.001$). Significance of difference between saccharin-

treated wild type and saccharin-treated 7(-/-), $*p < 0.05$ using a two-way ANOVA followed by a Bonferroni post hoc test.

Fig. 5.

Nicotine reduces L-dopa-induced AIMs in $\alpha 7(-/-)$ mice but not wild type mice after 5 wk of treatment. Lesioned mice were injected with L-dopa (3 mg/kg) plus benserazide (15 mg/kg) and given nicotine (nic) in the saccharin (sac)-containing drinking water for 5 wk. The daily time course of the nicotine-mediated reduction in total AIMs is provided on the left. The effect of nicotine on total, oral, axial and forelimb AIMs is shown in the panels on the right. The mice were subdivided into two groups, those with moderate and those with high AIM scores. Values represent the mean $\pm$ SEM of 3–6 in the moderate AIMs group and 8–10 in the high AIMs group. Where the SEM is not shown, it fell within the symbol. Significance of difference between the $\alpha 7(-/-)$ saccharin-treated and the $\alpha 7(-/-)$ nicotine-treated mice, * $p < 0.05$, ** $p < 0.01$; significance of difference between saccharin-treated wild type and saccharin-treated $\alpha 7(-/-)$ mice, # $p < 0.05$, ### $p < 0.001$ using two-way ANOVA followed by a Bonferroni post hoc test.

Fig. 6.

Nicotine more prominently reduces L-dopa-induced AIMs in $\alpha 7(-/-)$ mice than wild type mice after 14 wk of treatment. Mice were treated as described in the legend to Fig. 5. The daily time course of the nicotine-mediated reduction in total AIMs is provided on the left. The effect of nicotine on total, oral, axial and forelimb AIMs is shown in the panels on the right. The mice were subdivided into two groups, those with moderate and those with high AIM scores. Values represent the mean $\pm$ SEM of 3–6 in the moderate AIMs group and 8–10 in the high AIMs group. Significance of difference between saccharin (sac)-treated wild type and nicotine (nic)-treated wild type mice, $\$p < 0.05$, $\$\$p < 0.01$, $\$\$\$p < 0.001$; significance of difference between saccharin-treated $\alpha 7(-/-)$ and nicotine-treated $\alpha 7(-/-)$ mice, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; significance of difference between saccharin-treated wild type and saccharin-treated $\alpha 7(-/-)$, $\#p < 0.05$, $\#\#p < 0.01$ using two-way ANOVA followed by a Bonferroni post hoc test.

Fig. 7.

Nicotine reduces L-dopa-induced AIMs to a similar extent in $\alpha 7(-/-)$ mice and wild type mice after 23 wk of treatment. Mice were treated as described in the legend to Fig. 5. The daily time course of the nicotine-mediated reduction in total AIMs is provided on the left. The effect of nicotine on total, oral, axial and forelimb AIMs is shown in the panels on the right. The mice were subdivided into two groups, those with moderate and those with high AIM scores. Values represent the mean $\pm$ SEM of 3–6 in the moderate AIMs group and 8–10 in the high AIMs group. Where the SEM is not shown, it fell within the symbol. Significance of difference between saccharin (sac)-treated wild type and nicotine (nic)-treated wild type mice, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$ \$ \$}p < 0.001$; significance of difference between saccharin-treated $\alpha 7(-/-)$ and nicotine-treated $\alpha 7(-/-)$ mice, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ using two-way ANOVA followed by a Bonferroni post hoc test.

Table 1

Parkinsonian ratings in unilateral 6-OHDA-lesioned wild type and knockout mice. All mice were rated two wk after 6-OHDA lesioning for the forelimb asymmetry test, a measure of unilateral motor dysfunction. Animals were placed in a transparent cage and evaluated over a 3 min period for forepaw placement against the walls of the cage. Each value represents the mean $\pm$ SEM of 18–27 mice.

Genotype	Impaired forelimb use (%)
4 wild type	30.9 $\pm$ 1.1
4(–/–)	31.9 $\pm$ 2.8
7 wild type	31.5 $\pm$ 4.7
7(–/–)	34.3 $\pm$ 3.3

Table 2

No change in baseline L-dopa-induced AIM scores in saccharin-treated 4(−/−) nAChR compared to wild type mice. 4 nAChR null mutant mice and their wild type littermates were lesioned with 6-OHDA. Three wk later they were injected once daily with L-dopa (3 mg/kg) plus benserazide (15 mg/kg), as depicted in the timeline in Fig. 1. The data were obtained in wk 10, 15 and 25 of the saccharin/nicotine treatment phase. Mice were evaluated for oral, axial, forelimb and total AIMs for 1 min every 15 min over a 2-h period. Data shown are for the saccharin-treated mice only. Values represent the mean ± SEM of 4–6 mice.

Group	AIM type	Genotype	AIM score		
			Wk 10	Wk 15	Wk 25
Moderate AIM scores	Total	4 (+/+)	30.2 ± 3.2	31.0 ± 3.9	27.3 ± 1.8
		4 (−/−)	30.3 ± 2.1	32.0 ± 5.0	27.7 ± 2.8
	Oral	4 (+/+)	10.5 ± 0.5	12.3 ± 0.3	10.8 ± 1.3
		4 (−/−)	10.7 ± 1.1	11.8 ± 1.1	11.0 ± 0.6
	Axial	4 (+/+)	6.0 ± 2.0	5.8 ± 3.3	4.0 ± 1.8
		4 (−/−)	7.2 ± 1.2	6.0 ± 2.2	5.5 ± 1.9
	Forelimb	4 (+/+)	13.8 ± 2.1	13.0 ± 1.1	12.5 ± 1.3
		4 (−/−)	12.5 ± 1.2	14.2 ± 2.0	11.2 ± 0.9
High AIM scores	Total	4 (+/+)	68.5 ± 17.3	63.5 ± 13.6	51.0 ± 9.6
		4 (−/−)	64.0 ± 7.0	49.2 ± 7.2	46.0 ± 5.1
	Oral	4 (+/+)	20.0 ± 3.2	16.8 ± 3.3	11.8 ± 0.3
		4 (−/−)	16.8 ± 1.5	14.8 ± 1.7	12.0 ± 0.5
	Axial	4 (+/+)	22.8 ± 8.9	24.3 ± 6.0	20.8 ± 4.5
		4 (−/−)	22.8 Ch 3.5	17.2 ± 3.3	18.0 ± 3.0
	Forelimb	4 (+/+)	25.8 ± 7.0	22.5 ± 5.8	18.5 ± 5.0
		4 (−/−)	24.4 ± 2.9	17.2 ± 2.9	16.0 ± 2.4

Table 3

Plasma cotinine levels in lesioned wild type and knockout mice treated with nicotine. Unilateral 6-OHDA-lesioned L-dopa-treated wild type and null mutant mice were given saccharin without and with nicotine (300 µg/ml) in the drinking water. Plasma cotinine levels shown were measured 2 to 3 months after the start of nicotine treatment, and are representative of the levels throughout the study. Cotinine levels were not detected in saccharin-treated mice. Values represent the mean $\pm$ SEM of 10–17 mice.

nAChR subunit deletion	Group	Cotinine level (ng/ml)
4	Wild type	384 $\pm$ 110
	Null mutant	350 $\pm$ 71
7	Wild type	280 $\pm$ 71
	Null mutant	301 $\pm$ 77

Table 4

Several nAChR subtypes play a role in L-dopa-induced Aims

nAChR subtype	Baseline L-dopa-induced Aims		Nicotine reduces L-dopa-induced Aims		Role in L-dopa-induced Aims
	Wild type	Knockout	Wild type	knockout	
2 [#]	No change		Yes	No	Yes
6 ^{&}	No change		Yes	No	Yes
4	No change	No change	Yes	No	Yes
7	No change		Yes	Yes	Yes

[#]Taken from [34].

[&]Taken from [35].