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Evidence for a role for α6* nAChRs in L-dopa-induced dyskinesias using parkinsonian α6* nAChR gain-of-function mice

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Abstract

L-Dopa-induced dyskinesias (LIDs) are a serious side effect of dopamine replacement therapy for Parkinson's disease. The mechanisms that underlie LIDs are currently unclear. However, preclinical studies indicate that nicotinic acetylcholine receptors (nAChRs) play a role, suggesting that drugs targeting these receptors may be of therapeutic benefit. To further understand the involvement of α6β2* nAChRs in LIDs, we used gain-of-function α6* nAChR (α6L9S) mice that exhibit a 20-fold enhanced sensitivity to nAChR agonists. Wildtype (WT) and α6L9S mice were lesioned by unilateral injection of 6-hydroxydopamine (6-OHDA, 3 μg/ml) into the medial forebrain bundle. Three to 4 wk later, they were administered L-dopa (3 mg/kg) plus benserazide (15 mg/kg) until stably dyskinetic. L-dopa-induced abnormal involuntary movements (AIMs) were similar in α6L9S and WT mice. WT mice were then given nicotine in the drinking water in gradually increasing doses to a final 300 μg/ml, which resulted in a 40% decline AIMs. By contrast, there was no decrease in AIMs in α6L9S mice at a maximally tolerated nicotine dose of 20 μg/ml. However, the nAChR antagonist mecamylamine (1 mg/kg ip 30 min before L-dopa) reduced L-dopa-induced AIMs in both α6L9S and WT mice. Thus, both a nAChR agonist and antagonist decreased AIMs in WT mice, but only the antagonist was effective in α6L9S mice. Since nicotine appears to reduce LIDs via desensitization, hypersensitive α6β2* nAChRs may desensitize less readily. The present data show that α6β2* nAChRs are key regulators of LIDs, and may be useful therapeutic targets for their management in Parkinson's disease.

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Keywords

Dyskinesia; L-dopa; nicotine; 6-hydroxydopamine; Parkinson's disease

1. Introduction

Long-term L-dopa use is complicated by the emergence of AIMs or dyskinesias, for which there are currently few treatments (Huot et al., 2011, Connolly and Lang, 2014). There is thus a critical unmet need for therapies to reduce LIDs. Preclinical studies suggest a compelling role for the nicotinic cholinergic system (Quik et al., 2014). Nicotine administration alleviated LIDs up to 60% in a variety of parkinsonian animal models, suggesting it may represent a useful treatment option (Quik et al., 2007, Bordia et al., 2008, Huang et al., 2011a).

Nicotine generally exerts its effects in the brain by acting at nicotinic acetylcholine receptors (nAChRs), of which there are several subtypes. The primary subtypes in the striatum, a region prominently affected in Parkinson's disease and linked to LIDs, are the α4β2*, α6β2* and α7 nAChRs. The asterisk indicates the possible presence of other subunits in the receptor complex (Millar and Gotti, 2009, Quik and Wonnacott, 2011). Two approaches have proved useful in delineating the nAChRs that mediate the nicotine-induced decline in LIDs. One of these involves the use of drugs targeting select nAChRs. Work with α7 nAChR agonists showed that administration of ABT-107 or AQW051 to monkeys led to ~60% decline in LIDs (Di Paolo et al., 2014, Zhang et al., 2014b). β2* nAChR agonists, which act at both α4β2* and α6β2* subtypes, also significantly reduced LIDs in parkinsonian rats and monkeys. Varenicline, ABT-089, ABT-894, TC-8831, as well as other TC-agonists, attenuated LIDs by 30-60% (Huang et al., 2011b, Johnston et al., 2013, Quik et al., 2013a, Zhang et al., 2013, Zhang et al., 2014a). Interestingly, the general nAChR antagonist mecamylamine also reduced LIDs to a similar extent as nicotine and nAChR agonists (Bordia et al., 2010). This latter finding led to the suggestion that agonists may reduce LIDs by a nAChR desensitization block, a mechanism through which agonists also modulate other behaviors (Picciotto et al., 2008, Buccafusco et al., 2009). The idea that LIDs are reduced because of a nAChR blockade is also consistent with a recent study which showed that ablation of striatal cholinergic interneurons, which results in a loss of acetylcholine, markedly reduced LIDs (Won et al., 2014).

Studies with genetically modified mice lend further support to the idea that multiple nAChRs are involved in the regulation of LIDs. Deletion of the α7 nAChR led to an increase in baseline LIDs, although it did not affect the antidyskinetic effect of nicotine (Quik et al., 2013b). By contrast, mice lacking β2* nAChRs, that is, both the α4β2* and α6β2* subtypes, exhibited a 50% decline in baseline LIDs. In addition, nicotine treatment no longer reduced LIDs in β2 null mutant mice. Selective subunit deletion of only the α4 nAChR subunit resulted in loss of the antidyskinetic effect of nicotine with no change in baseline LIDs. By contrast, deletion of only the α6 nAChR subunit led to a decline in baseline LIDs together with a loss of the antidyskinetic effect of nicotine. These latter findings suggest an important role for α6β2* nAChRs in LIDs (Quik et al., 2012).

The objective of the current study was to use gain-of-function α6L9S mice to further explore the role of α6β2* nAChRs in LIDs. These mice express an α6* nAChR subunit in which the Leu 9′ residue in the M2 transmembrane domain is mutated to a Ser (Drenan et al., 2008). This mutation results in an α6β2* nAChR channel hypersensitive to endogenous acetylcholine or nAChR agonists, with a consequent increase in dopaminergic function (Drenan et al., 2008, Drenan et al., 2010, Wang et al., 2014). In addition, transgenic mice expressing α6L9S nAChRs exhibit a variety of enhanced ambulatory behaviors, including walking, turning and rearing (Drenan et al., 2010). The present data using such transgenic mice further support a role for α6β2* nAChRs in LIDs.

2. Materials and methods

2.1 Animals and nigrostriatal lesioning

Gain-of-function α6L9S mice and their WT littermates were bred, raised and genotyped at Purdue University, as described (Drenan et al., 2008). Adult male mice (20-35g) were then shipped to SRI for lesioning, behavioral and biochemical studies. Upon arrival, mice were group housed in a room with controlled temperature and humidity, and a 12 h light/dark cycle. The mice had free access to food and water. After one wk of acclimation, the mice were lesioned by unilateral intracranial injection of 6-OHDA (Sigma-Aldrich Co., St. Louis, MO) into the right medial forebrain brain, as described (Lundblad et al., 2004, Lundblad et al., 2005, Huang et al., 2011a, Quik et al., 2012, Quik et al., 2013b). 6-OHDA (3 μg free base/μl in 0.9% saline containing 0.02% ascorbic acid) was stereotaxically injected under isofluorane anesthesia at the following site: anteroposterior, -1.2; lateral, -1.2; ventral, 4.75, relative to the bregma. The cannula was slowly lowered into the brain, with 6-OHDA delivered over a 2 min period. The cannula was maintained at the target site for an additional 2 min, followed by a 2 min removal period. Buprenorphine (0.3 mg/kg) was injected subcutaneously for post-operative pain management and a 0.5 ml aliquot of physiological saline to minimize dehydration. Following surgery, a 20% sucrose solution containing ground food pellets was placed at the bottom of the cage to assist feeding for 1 to 2 wk, as necessary.

All procedures were approved by the Institutional Animal Care and Use Committee in accordance with the NIH. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2 Behavioral measurements

Three weeks after 6-OHDA lesioning, mice were assessed for nigrostriatal damage using the forelimb use asymmetry test (cylinder test) (Fig. 1). Mice were placed singly in a transparent cylinder and rated for 3 min for exploratory activity by a blinded rater (Huang et al., 2011a, Quik et al., 2012, Quik et al., 2013b). Contacts with the container wall using the impaired forelimb (contralateral to the lesion) were expressed as % of total forelimb contacts.

Mice were then administered L-dopa (3 mg/kg) plus benserazide (15 mg/kg) (both from Sigma-Aldrich Co., St. Louis, MO) subcutaneously once daily 3 d per wk (Fig. 1), as described (Huang et al., 2011a, Quik et al., 2012, Quik et al., 2013b). Two wk later, they were assessed for L-dopa-induced AIMs. Briefly, mice were injected with L-dopa and placed in separate clear containers. Ten min after the injection they were scored individually for 1 min every 15 min over a 2 h period by a blinded rater. Each AIM subtype (oral, forelimb, and axial) was scored on a frequency scale ranging from 0 to 4 (0 = no AIMs; 1 = occasional AIMs displayed <50% of the observation time; 2 = sustained AIMs for >50% of the observation time; 3 = continuous AIMs; 4 = continuous AIMs not interruptible by external stimuli). Each of the AIM subtypes was also scored for amplitude designated as A or B, with “A” representing oral AIMs without tongue protrusion, forelimb AIMs without shoulder involvement, and axial AIMs with body twisting <60°. “B” represented oral AIMs with tongue protrusion, forelimb AIMs with shoulder involvement or axial AIMs with body twisting >60°. The total score per mouse at any time point was calculated as follows; 1A = 1, 1B = 2, 2A = 2, 2B = 4, 3A = 4, 3B = 6, 4A = 6, 4B = 8, with a score for any one component (axial, oral or forelimb) ranging from 0 to 8. Therefore, the maximum possible score for each mouse was 192 (max score per session = 24, with 8 sessions over the 2 h period).

2.3 Drug treatments

After 3 wk of L-dopa treatment when dyskinesias are stably expressed, α6L9S and WT mice were acclimated to 2% saccharin drinking solution for 2 d. Saccharin was necessary to mask the bitter of taste of nicotine (Fig. 1). The two genotypes were then divided into two groups each, with one receiving drinking water with only saccharin and the other saccharin-containing nicotine. The mean total dyskinesia scores were similar in all groups. For the WT mice, nicotine treatment was started at a dose of 25 μg/ml for 2 d, 50 μg/ml for 2 d, 100 μg/ml for 3 d, 200 μg/ml for 3 d and then 300 μg/ml, at dose at which the WT mice were maintained, as previously described (Huang et al., 2011a, Quik et al., 2012, Quik et al., 2013b). Previous work by others has shown that such a dosing regimen yields brain nicotine levels of approximately 1 μM, with smoking levels about 0.3 μM (Gaddnas et al., 2001, Matta et al., 2007).

The α6L9S mice were also given 25 μg/ml nicotine in the drinking water for 2 days, 50 μg/ml for 3 d, followed by 100 μg/ml. However, 5 of the 20 α6L9S mice died at this dose after 7 d of treatment. The nicotine was therefore decreased to 75 μg/ml for 7 days with 3 more deaths, followed by a reduction to 50 μg/ml with 2 deaths, followed by a reduction to 25 μg/ml with 2 more deaths, with only 1 mouse death at 20 μg/ml. The enhanced sensitivity of α6L9S to nicotine is consistent with previous behavioral and electrophysiological studies which demonstrated a ∼20 times greater sensitivity to nicotine (Drenan et al., 2008).

The mouse weights were not affected by nicotine treatment, although the weights of the α6L9S mice were somewhat lower than the WT littermates. Values (g) at wk 10 (white box in timeline) were as follows: WT saccharin 43 ± 2 (n = 10) and WT nicotine 37 ± 2 (n = 10); α6L9S saccharin 34 ± 1 (n = 10) and α6L9S nicotine 31 ± 1 (n = 7).

2.4 Tissue preparation

Mice were killed by cervical dislocation 45 min after L-dopa administration. The brains were quickly removed and quick frozen in isopentane on dry ice and stored at -80°C. When required, 8 μm sections were cut at -15°C in a cryostat (Leica Microsystems Inc., Deerfield, IL), thaw mounted onto poly-L-lysine coated slides, dried, and stored at -80°C.

2.5. Binding studies

Striatal dopamine transporter binding was performed using ^{125}I-3β-(4-iodophenyl)tropane-2β-carboxylic acid isopropyl ester (^{125}I-RTI-121, specific activity 2200 Ci/mmol; PerkinElmer Life and Analytical Sciences, Waltham, MA) as described (Quik et al., 2003). This technique was used because it provides a quantitative assessment of dopamine transporter levels (Quik et al., 2003). To measure transporter levels, the sections were first pre-incubated at room temperature for two 15 min periods in buffer containing 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, and 5 mM KCl. Next, they were incubated for 2 h in the same buffer also containing 0.025% bovine serum albumin (BSA), 1 μM fluoxetine, and 50 pM ^{125}I-RTI-121. Nonspecific binding was determined in the presence of the uptake inhibitor nomifensine (100 μM). Slides were then washed four times for 15 min in ice-cold buffer, once for 10 s in ice-cold water and air dried.

Striatal α4β2* nAChR levels were determined using ^{125}I-epibatidine (specific activity, 2200 Ci/mmol; PerkinElmer Life and Analytical Sciences, Waltham, MA) in the presence of 10^{-7} μM of the α6β2* nAChR blocker α-conotoxinMII (α-CtxMII), as described (Quik et al., 2003). Briefly, the thawed sections were first pre-incubated for 15 min in binding buffer containing 50 mM Tris, pH 7.0, 120 mM NaCl, 5 mM KCl, 2.5 mM CaCl2, and 1.0 mM MgCl2 and α-CtxMII. This was followed by 40 min incubation in buffer also containing 0.03 nM ^{125}I-epibatidine with α-CtxMII. Nicotine (100 μM) was used to determine nonspecific binding. To terminate binding, the slides were washed twice for 5 min in ice-cold buffer and once for 10 s in ice-cold deionized water and air dried.

Striatal a6p2* nAChRs binding levels were measured using ^{125}I-α-conotoxinMII binding (^{125}I-α-CtxMII; specific activity, 2200 Ci/mmol) as previously described (Quik et al. 2003). The thawed sections were first pre-incubated for 15 min in binding buffer containing 144 mM NaCl, 1.5 mM KCl, 2 mM CaCl2, 1 mM MgSO4, 20 mM HEPES, 1 mM PMSF (phenylmethylsulfonyl fluoride) and 0.1% BSA, pH 7.5. Following pre-incubation, the slides were incubated for 1 h in binding buffer which also contained 0.5% BSA, 5 mM EDTA, 5mM EGTA, 10 μg/ml each of aprotinin, leupeptin and pepstatin A, and 0.5 nM ^{125}I-α-CtxMII. Nicotine (100 μM) was used to determine nonspecific binding. The binding assay was terminated by washing the slides for 10 min at 22°C in binding buffer, 10 min in ice-cold binding buffer, twice for 10 min in ice-cold 0.1 × binding buffer, and twice for 10 s in ice-cold deionized water.

After air drying, slides were exposed to Kodak MR Film (Easterman Kodak Co., Rochester, NY) as needed along with ^{125}I-microscale standards (American Radiolabeled chemicals, Inc., Saint Louis, MO).

2.6 Data analyses

For quantitation of the autoradiograms, optical density measurements were assessed using the ImageQuant system (GE Healthcare, Little Chalfont, Buckinghamshire, UK). These values were converted to fmol/mg tissue using standard curves generated from ^{125}I-standards. The optical density readings of the samples fell within the linear range of the standards. Data analyses were done with GraphPad Prism® (GraphPad Software, Inc, San Diego, CA) using analysis of variance (ANOVA) followed by the appropriate post hoc test. A level of 0.05 was considered significant.

3. Results

3.1. Nicotine reduces L-dopa-induced AIMs in WT but not α6L9S mice

The results in Fig. 2 show the effect of nicotine on L-dopa-induced AIMs in WT and α6L9S mice over a 10 wk period. L-dopa-induced AIMs were similar in the WT and α6L9S mice at the start of the nicotine treatment regimen with values of 26.1 ± 4.10 (n=21) for WT and 21.7 ± 2.15 (n = 31) for α6L9S. The variability in AIMs was similar to that observed in our previous studies; the basis for this variability is not clear but does not appear to relate to the size of the lesion (Huang et al., 2011a, Quik et al., 2012, Quik et al., 2013b). Nicotine treatment led to a gradual decrease in total AIM scores in WT mice, which was significant at wk 8 and 10. By contrast, nicotine treatment had no effect on AIM scores in α6L9S mice. Since our previous studies demonstrated differential effects of nicotine in mice with low and higher AIM scores, mice were subdivided into two such groups (Huang et al., 2011a, Quik et al., 2012, Quik et al., 2013b). The data in the lower panels of Fig. 2 show that the results were comparable to those in the all mice group.

Fig. 3 depicts effects on the various L-dopa-induced AIM components, that is, oral, axial and forelimb AIMs in all mice, as well as in mice with low and higher AIM scores. The different AIM subtypes were similarly expressed in saccharin-treated WT and α6L9S mice. Nicotine treatment reduced AIMs in WT mice mainly via a decrease in oral AIMs, with a lesser effect on forelimb AIMs. Significant reductions ($p < 0.001$) were observed in oral AIMs in the all mice group (Fig. 3 top panel), as well as in mice with low ($p < 0.01$) and higher ($p < 0.01$) AIM scores (Fig. 3 lower panels). The nicotine-mediated reduction ($p < 0.01$) in forelimb AIM was observed only in the higher AIMs group (Fig 3 bottom). There was no effect of nicotine treatment on any AIM subtype in the α6L9S mice in any group. No axial AIMs were observed in the current study, possibly because total AIMs were not that severe in these experiments.

Our previous studies demonstrated that AIMs peaked ~60 min after L-dopa administration with an overall duration of effect of ~2 h (Huang et al., 2011a, Quik et al., 2012, Quik et al., 2013b). A similar pattern of AIMs expression was observed for α6L9S mice (Fig. 4). Again, nicotine treatment significantly reduced AIMs expression in WT mice but not α6L9S mice.

Parkinsonism was measured using the forepaw placement or cylinder test. In vehicle treated unilateral 6-OHDA lesioned WT mice, a decline was observed in contralateral forepaw use (37.3 ± 2.2%, n = 17), with similar results in unilaterally lesioned α6L9S mice (36.9 ± 2.3%, n = 25).

3.2 The nAChR blocker mecamylamine decreases L-dopa-induced AIMs in both WT and α6L9S mice

Our earlier studies had shown that the nAChR blocker mecamylamine also reduced L-dopa-induced AIMs (Bordia et al., 2010). These findings led to the suggestion that nicotine decreases L-dopa-induced AIMs via a desensitizing block. The present experiments were done to determine if mecamylamine also attenuated L-dopa-induced AIMs in mice expressing hypersensitive α6β2* nAChRs. The saccharin-treated WT and α6L9S mouse groups were injected 10 min before L-dopa administration for 1 or 2 d with saline or 1 mg/kg mecamylamine. This dose was used as previous studies had shown that it is effectively reduces locomotor activity in α6L9S mice (Drenan et al., 2008), and WT mice (Bhutada et al., 2010, Biala and Staniak, 2010). Mecamylamine injection significantly reduced total AIMs and the individual AIM components in both WT mice and α6L9S mice, with the most pronounced effects after 2 d of treatment (Fig. 5). The observation the nAChR blocker mecamylamine reduced AIMs despite a lack of effect of nicotine suggests that nicotine may no longer be able to desensitize hypersensitive α6β2* nAChRs. Such an interpretation would suggest that the antidyskinetic effect of nicotine is mediated primarily via α6β2* nAChRs.

3.3 Nicotine treatment leads to an improvement in dopamine transporter levels in 6-OHDA lesioned WT and α6L9S mice

Striatal dopamine transporter levels were measured using ^{125}I-RTI-121 binding on the intact and lesioned side of WT and α6L9S mice treated with nicotine or saccharin (Fig. 6). Dopamine transporter levels were similar in saccharin-treated WT and α6L9S mice. Nicotine treatment alone did not alter dopamine transporter levels on the intact side of WT mice, as previously shown (Huang et al., 2011a, Quik et al., 2012, Quik et al., 2013b). Nicotine administration also did not affect transporter levels in intact striatum of α6L9S mice. Lesioning alone decreased striatal ^{125}I-RTI-121 binding by 30% in WT, consistent with previous findings (Quik et al., 2003). Lesioning resulted in a similar decline in α6L9S mice, indicating that genetic manipulation of the α6 subunit did not influence the extent of nigrostriatal damage. Interestingly, long term nicotine treatment led to improved transporter levels in both WT and α6L9S mice comparable to those on the intact side. These findings suggest that nicotine may induce sprouting of nigrostriatal dopamine terminals, with a consequent restoration of dopamine transporter levels.

3.4 Low dose nicotine is sufficient to regulate striatal α4β2* but not α6β2* nAChRs in α6L9S mice

To evaluate whether the low dose of nicotine used in the drinking water of α6L9S mice modulated nAChR expression, we measured α4β2* nAChRs (Fig. 7). These receptors are well known to up-regulate with long term nicotine treatment in WT mice (Marks et al., 1992, Pauly et al., 1996, Lai et al., 2005). α4β2* nAChR levels were determined by measuring ^{125}I-epibatidine in the presence of α-CtxMII to block binding to α6β2* nAChRs. The results show that α4β2* nAChR levels were similar in WT and α6L9S mice. 6-OHDA lesioning did not affect α4β2* nAChR binding levels, most likely because the majority of α4β2* nAChR in the striatum (80-85%) are not located on the lesioned nigrostriatal dopamine terminals (Quik et al., 2003). As expected, long term nicotine treatment increased α4β2* nAChRs in the intact and lesioned striatum of WT mice (Lai et al., 2004). Notably, there was also an increase in α4β2* nAChR binding levels in the α6L9S mice. These data indicate that the low dose of nicotine (20 μg/ml) used to treat the α6L9S mice leads to changes in striatal nAChR expression.

In addition, experiments were done to determine whether nicotine treatment affected α6β2* nAChRs. α6β2* nAChRs were decreased on the lesioned side in both WT and α6L9S mice (Fig. 8), as expected since these are expressed on dopamine terminals in the striatum (Quik et al., 2003). Nicotine treatment down-regulated α6β2* nAChRs on the intact side of WT, consistent with previous studies (Lai et al., 2005). Nicotine treatment did not affect α6β2* nAChRs in α6L9S mice. With respect to combined lesioning and nicotine treatment, α6β2* nAChR levels were similar on the intact and lesioned side in WT mice. This result again suggests that the molecular integrity of dopamine terminals is restored/enhanced with nicotine treatment, in agreement with the DAT results in Fig. 6. By contrast, α6β2* nAChR levels remained low in the α6L9S mice, although α4β2* nAChRs were upregulated under the same treatment.

4. Discussion

The present study provides further evidence for a role for α6β2* nAChRs in L-dopa-induced AIMs using gain-of-function α6L9S mice, a unique model exhibiting enhanced α6* receptor responsiveness. Consistent with previous studies, the present findings show that long-term nicotine treatment decreased L-dopa-induced AIMs in WT mice (Huang et al., 2011a, Quik et al., 2012). By contrast, no such decline was observed in mice expressing hypersensitive α6β2* nAChRs. Despite the lack of effect of the agonist nicotine on L-dopa-induced AIMs in α6L9S mice, the nAChR antagonist mecamylamine reduced AIMs in α6L9S mice to a similar extent as in WT mice, with these latter results in line with previous work in rats (Bordia et al., 2010). Since nicotine-mediated effects on behavior have been postulated to occur through nAChR desensitization, these data suggest that nicotine failed to desensitize α6L9S nAChRs. The present findings provide support for the idea that nAChR-mediated declines in LIDs occur via desensitization and that α6β2* nAChRs are involved.

α6L9S mice have proved very useful for delineating a role for α6* nAChRs in regulating dopaminergic function (Drenan et al., 2008, Drenan et al., 2010, Engle et al., 2013, Wang et al., 2014). These mice express an α6* nAChR in which the Leu 9′ residue in the M2 domain of the α6 subunit is modified to a Ser (Drenan et al., 2008). This change results in an α6* nAChR that is ~20 more sensitive to acetylcholine. This enhanced sensitivity is associated with an increase in dopamine neuron excitability in dopaminergic brain regions including the striatum, olfactory tubercle and ventral tegmental area (Drenan et al., 2008, Drenan et al., 2010, Wang et al., 2014). In addition, there was augmented ^{3}H-dopamine release from synaptosomes and increased evoked extracellular dopamine levels in slices from α6L9S compared to WT mice (Drenan et al., 2008, Drenan et al., 2010, Wang et al., 2014). HPLC measurements also demonstrated elevated levels of dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid in dopaminergic areas from α6L9S mice compared to WT, while western blotting showed an increase in tyrosine hydroxylase (Wang et al., 2014). This enhanced dopaminergic function, in turn, led to altered behavioral responses including increased walking, turning and rearing in α6L9S compared to WT mice that may be linked to changes in nigrostriatal function (Drenan et al., 2008, Drenan et al., 2010). Heightened dopaminergic function in the mesolimbic system has also been suggested from studies showing that α6L9S mice are more sensitive to the rewarding effects of alcohol (Powers et al., 2013). Since LIDs are thought to arise because of enhanced dopaminergic tone, an increase in their expression might have been expected in α6L9S mice. However, the similarity in LIDs in WT and α6L9S mice suggests that compensatory mechanisms developed to curtail their intensity in α6L9S mice. This is not unexpected since LIDs are modulated by numerous neurotransmitters, including the serotonergic, glutamatergic, opioid, noradrenergic and GABAergic systems (Huot et al., 2013).

Not only do α6L9S mice exhibit enhanced spontaneous motor activities and increased responsiveness to the rewarding effects of alcohol, but they are also much more sensitive to the effects of administered nicotine. For instance, low dose nicotine (0.02 to 0.15 mg/kg ip) markedly increased locomotor activity in α6L9S mice, while these doses had no effect in WT mice (Drenan et al., 2008). This elevated motor responsiveness was blocked by mecamylamine, indicating the effect was nAChR-mediated. These enhanced nicotine-mediated behavioral changes correlated well with nicotine-mediated hyper-responsiveness at the cellular level.

Evidence for enhanced sensitivity to nicotine is also readily evident in the current study, with the α6L9S mice being much less tolerant to a nicotine administration regimen that presented no problems in WT mice. Typically, nicotine dosing to WT mice is started at 25 μg/ml with a gradual increase to 300 μg/ml with no detectable adverse effects (Sparks and Pauly, 1999, Lai et al., 2005, Huang et al., 2011a). However, when α6L9S mice were subjected to a similar nicotine treatment regimen, 25% mortality was observed at 100 μg/ml nicotine. After several dose reductions, only a dose of 10 μg/ml was not associated with mortality.

Our previous results had shown that nicotine treatment reduced L-dopa-induced AIMs (Bordia et al., 2010, Huang et al., 2011b) and that mecamylamine administration also decreased their occurrence (Bordia et al., 2010). This somewhat unexpected observation that both a nAChR agonist and antagonist ameliorated AIMs led to the suggestion that nicotine exerted its effect via a desensitization blockade. Such an interpretation is consistent with other studies which indicate that nicotine modulates behaviors, such as cognition, addiction and depression, via a receptor activation followed by desensitization (Buccafusco et al., 2009, Mineur and Picciotto, 2010). The observation that mecamylamine still reduced L-dopa-induced AIMs in α6L9S mice would suggest that α6L9S receptors can still be blocked by an antagonist although they do not appear to be desensitized in response to nicotine exposure. Such an interpretation suggests that the antidyskinetic effect of nicotine is mediated via α6β2* nAChRs, at least in α6L9S mice.

A question that arises is whether the lack of effect of nicotine on L-dopa-induced AIMs in α6L9S mice may be due to the low dose of nicotine administered to the transgenic mice. The present data suggest this is unlikely. Our receptor studies show that α4β2* nAChRs are up-regulated in the striatum of WT mice, consistent with previous work (Marks et al., 1983, Pauly et al., 1996, Lai et al., 2005, Bordia et al., 2010). A significant receptor increase was also observed in α6L9S mice, attesting to the effectiveness of the low dose nicotine in the brain. Second, studies involving measurement of the dopamine transporter show that elevated transporter levels were observed in the striatum of both lesioned WT and α6L9S mice following either dose of nicotine. This provides further evidence for efficacy of the lower nicotine dose in the α6L9S mice.

The present data show that long term nicotine treatment increases striatal α4β2* nAChR levels (Marks et al., 1992, Lai et al., 2005, Srinivasan et al., 2014), while α6β2* nAChRs are decreased, as previously shown (Lai et al., 2005, Perry et al., 2007). Studies to understand the functional consequences of these opposing changes in nAChR levels with nicotine treatment show that nAChR-mediated dopamine release is deceased with chronic nicotine treatment (Quik et al., 2012, Bordia et al., 2013). This has been attributed to nicotine-induced α4β2* nAChR desensitization and the observed decline in α6β2* nAChRs (Marks et al., 1993, Bordia et al., 2013). In addition to the idea that chronic nicotine administration acts by decreasing dopamine release via striatal nAChR desensitization and down-regulation, other molecular changes may also be involved. It has been shown that nicotine treatment alters D1 and D2 receptor characteristics and modulates the function of striatal interneurons and medium spiny neurons (Garcia-Montes et al., 2012). In addition, nicotine administration affects GABA responsiveness in the substantia nigra and consequently nigrostriatal dopaminergic and striatal glutamatergic function (Xiao et al., 2009). Thus nicotine may act via multiple cellular and molecular mechanisms throughout the brain to diminish dopamine release and consequently reduce LIDs.

The receptor autoradiography data show that nigrostriatal damage results in a significant decline in striatal α6β2* nAChRs. By contrast, α4β2* nAChRs are not appreciably reduced in the current study probably due to the relatively small lesion. This apparent lack of effect on α4β2* nAChR relates the fact that only a small proportion of α4β2* nAChRs are present on nigrostriatal dopamine terminals with the majority present on other neurons in the striatum (Quik et al., 2003). However, dopamine release studies show that small declines in striatal α4β2* nAChR levels may be associated with significant losses in α4β2* nAChR-mediated function (Quik et al., 2003). Thus, drugs targeting α6β2* or α4β2* nAChRs may be of similar value for therapeutic use.

The observation that nicotine dosing elevates dopamine transporter levels in the lesioned striatum was somewhat unexpected. This increased DAT is most likely on or within dopamine nerve terminals since DAT is only associated with dopaminergic neurons in the striatum (Seeman and Niznik, 1990, Miller et al., 1999). The enhanced DAT levels may be due to the relatively long term nicotine treatment regimen used in the present study (6 months). This idea stems from studies showing that nAChR agonists and antagonists can modulate neuritic outgrowth in neuronal cells in culture (Chan and Quik, 1993, Zheng et al., 1994, Erskine and McCaig, 1995, Owen and Bird, 1995, Coronas et al., 2000). In addition, nicotine administration to rats increased fibroblast growth factor mRNA and protein, as well as nerve growth factor levels in rodent brain (Belluardo et al., 2000, Jonnala et al., 2002). Of more direct relevance to the current study, nicotine exposure increased dendritic arborization and soma size in mouse mesencephalic dopaminergic neurons in culture (Collo et al., 2013). Thus the nicotine-mediated increase in dopamine transporter levels in the striatum of WT and α6L9S mice may be due to enhanced outgrowth of dopaminergic neurites that occurs when the system is compromised by lesioning.

In summary, the current studies using mice expressing gain-of function α6L9S nAChR further implicate α6β2* nAChRs in LIDs. In addition, the data suggest that α6β2* nAChR blockade may be a useful strategy for reducing LIDs. Since α6β2* nAChRs are expressed relatively selectively on dopaminergic neurons in the brain, the use of drugs targeting these receptors may yield beneficial results with a minimum of side effects.

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Abbreviations

AIMs	abnormal involuntary movements
ANOVA	analysis of variance
α-CtxMII	α-conotoxinMII
LIDs	L-dopa-induced dyskinesias
nAChRs	nicotinic receptors
6-OHDA	6-hydroxydopamine
^{125}I-RTI-121	^{125}I-3β-(4-iodophenyl)tropane-2β-carboxylic acid isopropyl ester
WT	wildtype
*	denotes the possible presence of other subunits in the receptor complex

1. Chronic nicotine treatment reduces AIMs in WT but not α6L9′S mice.
2. The nAChR antagonist mecamylamine reduces AIMs in both WT and α6L9′S mice.
3. Nicotine may decrease AIMs via desensitization blockade of α6 nAChRs.
4. α6* nAChR antagonists may be useful for reducing LIDs.

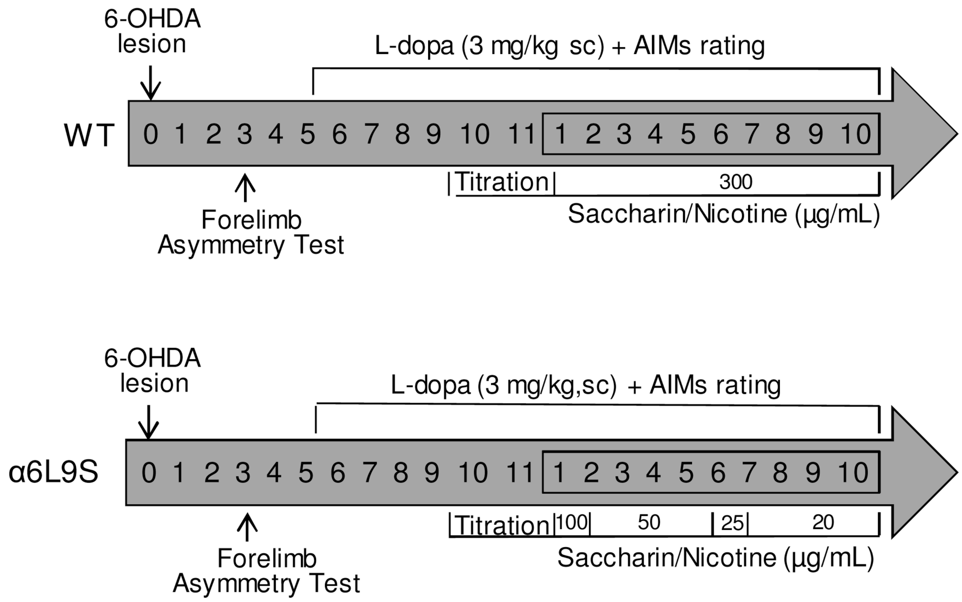

Fig. 1.

Treatment schedule. α6L9S mice and their WT littermates were unilaterally lesioned with 6-OHDA. The forelimb asymmetry test was then done to evaluate motor deficits. The mice were subsequently rendered dyskinetic by once daily injection of L-dopa plus benserazide for 3 wk. They were rated for L-dopa-induced AIMs throughout the study. At wk 9 (grey box) all mice were acclimated for 2-3 d to 2% saccharin solution after which they were either continued on saccharin or given nicotine as indicated in the timeline. The white box represents the number of wk of nicotine treatment. L-dopa and nicotine treatments were continued until the time of death.

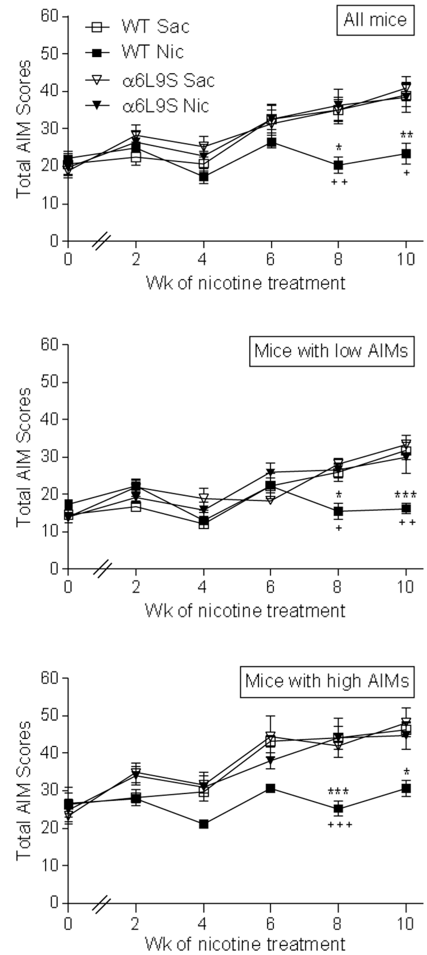

Fig. 2.

Weekly time course showing a decrease in L-dopa-induced AIM scores with nicotine treatment in WT but not α6L9S. L-dopa-treated lesioned WT and α6L9S mice were treated with saccharin (Sac) or nicotine (Nic) as detailed in the timeline in Fig. 1. The data shown are for 10 wk of treatment, for all mice (top), mice expressing low AIMs (middle) and mice expressing high AIMs (bottom). Values are the mean ± SEM of 3-10 mice. Significance of difference from the WT saccharin-treated group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; from the α6L9S nicotine-treated group, $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ using two-way ANOVA followed by a Bonferroni *post hoc* test.

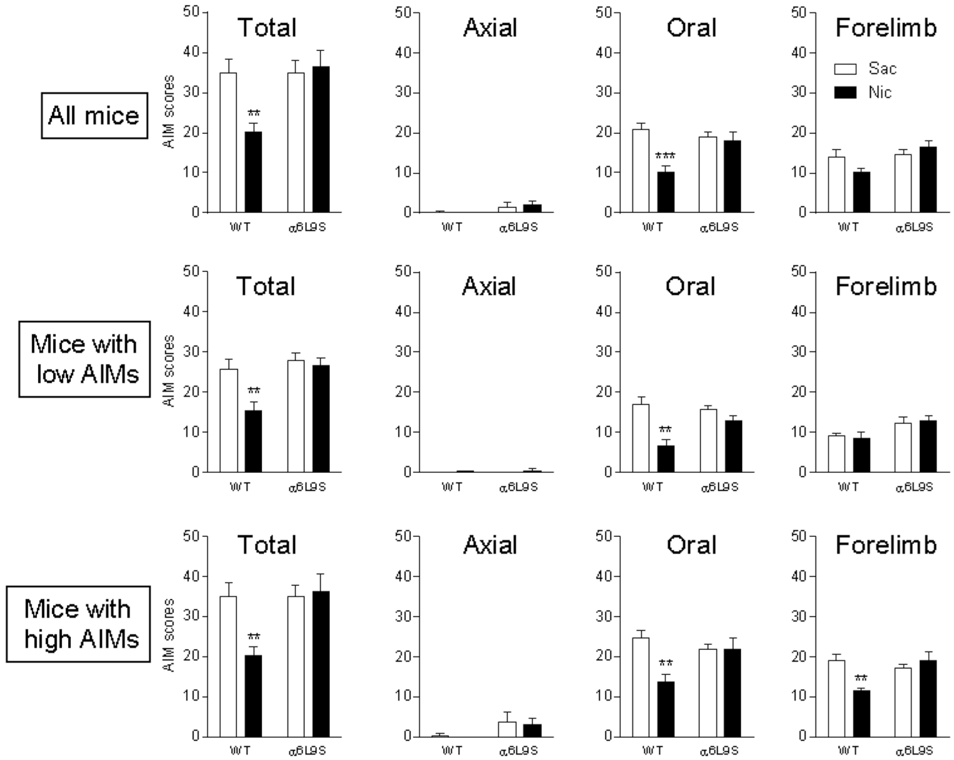

Fig. 3.

Nicotine treatment decreased various L-dopa-induced AIMs components in WT but not α6L9S mice. Saccharin and nicotine-treated WT and α6L9S mice treated were rated for axial, oral and forelimb AIMs, with total AIMs representing the sum of the three components. The values shown are at 8 wk of nicotine treatment for all mice (top panels), mice expressing low AIMs (middle panels) and mice expressing high AIMs (bottom panels). Values are the mean ± SEM of 3-10 mice. Significance of difference from the WT saccharin-treated group, **p < 0.01, ***p < 0.001 using two-way ANOVA followed by a Bonferroni *post hoc* test.

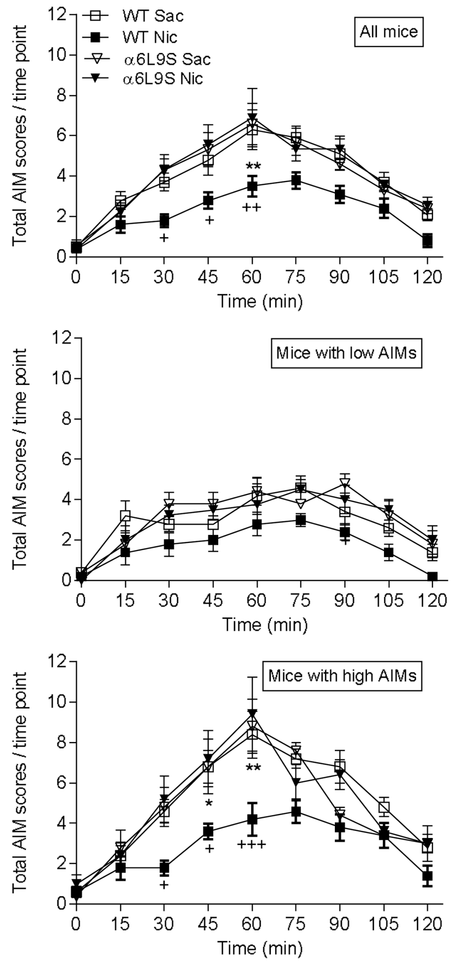

Fig. 4.

Hourly time course shows that nicotine treatment decreased L-dopa-induced AIMs throughout the treatment period in WT but not α6L9S mice. The data shown are for total L-dopa-induced AIMS for all mice (top), mice expressing low AIMs (middle) and mice expressing high AIMs (bottom) at 8 weeks of nicotine treatment. Values are the mean ± SEM of 3-10 mice. Significance of difference from the WT saccharin-treated group, $^{*}p < 0.05$, $^{**}p < 0.01$; from α6L9S nicotine-treated group, $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ using two-way ANOVA followed by a Bonferroni *post hoc* test.

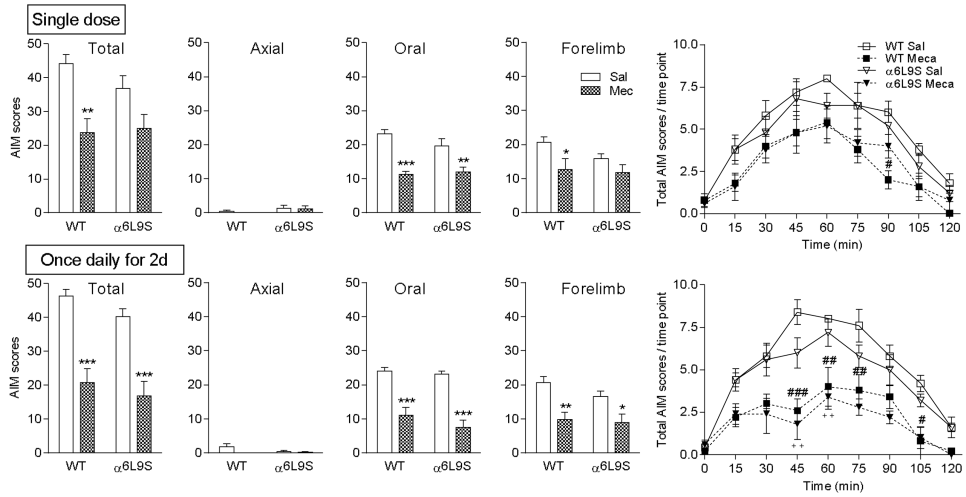

Fig. 5.

The general nAChR antagonist mecamylamine reduces AIMs in both WT and α6L9S mice. WT and α6L9S mice were injected with saline (Sal) or 1 mg/kg mecamylamine (Mec) 30 min before L-dopa for 1 or 2 days. Data shown are for a single injection (top panel) or two d of mecamylamine treatment (bottom panels). The hourly time is shown in the right panels. Values are the mean ± SEM of 5 mice per group. Significance of difference from the corresponding saline-treated group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; from WT saline-treated mice, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.01$; from α6L9S mecamylamine-treated mice, $^{++}p < 0.01$ using two-way ANOVA followed by a Bonferroni *post hoc*.

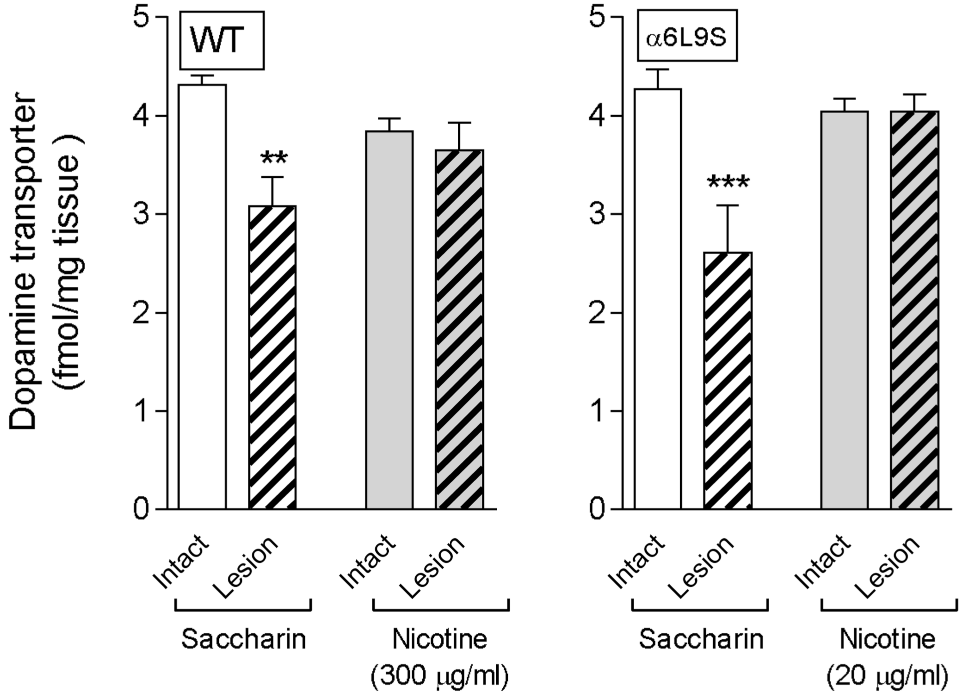

Fig. 6.

Nicotine treatment leads to an improvement in the dopamine transporter in 6-OHDA lesioned WT and α6L9S mice. 6-OHDA lesioning led to a decline in the dopamine transporter. By contrast, this decrease on the lesioned side was no longer observed in either WT and α6L9S mice with nicotine treatment. Values are the mean ± SEM of 7-10 mice per group. Significance of difference from the intact side of WT saccharin-treated mice, **p < 0.01, ***p < 0.01.

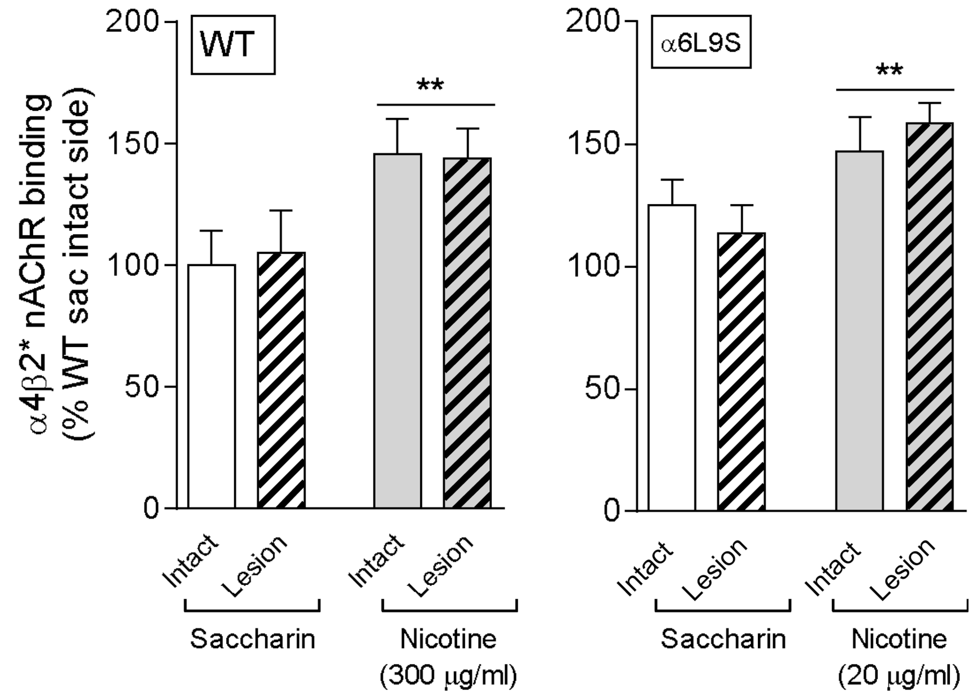

Fig. 7.

Low dose nicotine is sufficient to up-regulate α4β2* nAChRs in α6L9S mice. α4β2* nAChR levels were determined using ^{125}I-epibatidine autoradiography in the presence of α-CtxMII. Values are the mean ± SEM of 7-10 mice per group. Significant main effect of nicotine treatment, **$p < 0.01$.

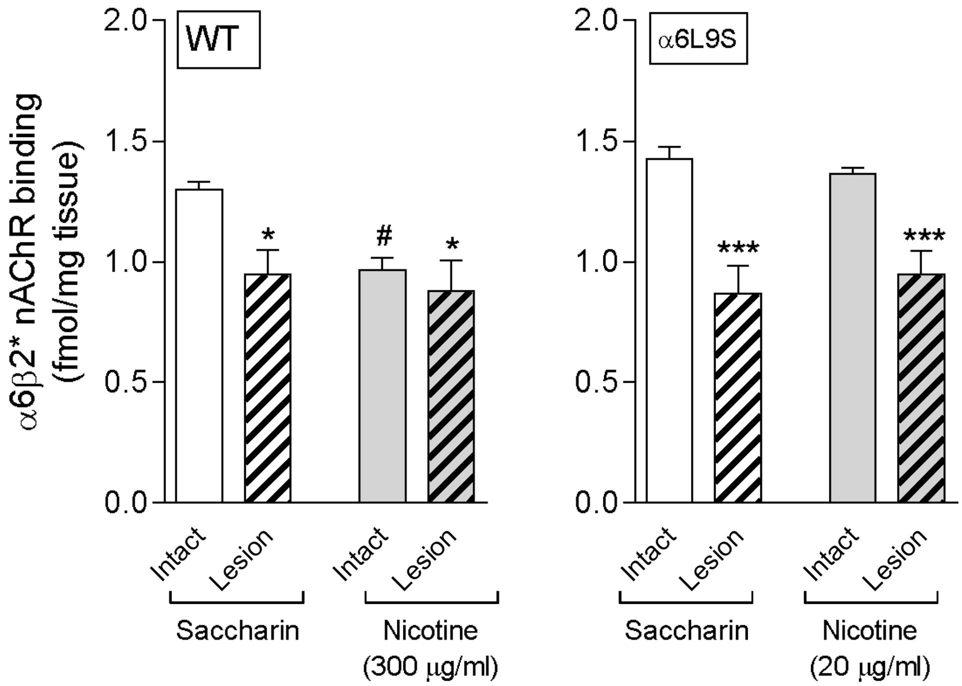

Fig. 8.

Effect of lesioning and nicotine treatment on α6β2* nAChRs in WT and α6L9S mice. α6β2* nAChR levels were determined using ^{125}I-α-CtxMII autoradiography. α6β2* nAChRs were decreased on the lesioned side, as expected since these are primarily expressed on dopamine terminals in the striatum. Nicotine treatment down-regulated α6β2* nAChRs on the intact side of WT. However, α6β2* nAChR levels were similar on the intact and lesioned side in WT mice, in agreement with the DAT levels in Fig. 6. Nicotine treatment did not affect α6β2* nAChRs in α6L9S mice. Values are the mean ± SEM of 7-10 mice per group. Significance of difference from the intact side, *$p < 0.01$, ***$p < 0.01$, $^{\#}p < 0.001$; from intact side of WT saccharin-treated mice.

Table 1

Summary of the effect of nicotine and mecamylamine on L-dopa- induced AIMs in α6L9S and α6 (-/-) mice. The present results (Figs. 2-5) show that both nicotine and mecamylamine treatments decreased L-dopa-induced AIMs by ~50% in the α6 WT mice. By contrast, mecamylamine but not nicotine decreased AIMs in α6L9S mice. These data suggest that nAChR drugs reduce AIMs by an antagonist action. We hypothesize that the lack of effect of nicotine is due to its inability to desensitize hypersensitive α6L9S nAChRs, at least at the concentrations used in this study. Our previous work with α6 (-/-) mice had shown that baseline L-dopa-induced AIMs were reduced with no further decline with nicotine treatment (Quik et al., 2012). These combined observations suggest that α6* nAChRs play a major role in the expression of L-dopa-induced AIMs.

Treatment	**Total L-dopa-induced AIMs (% saccharin WT)**			
	α6 WT	**α6L9S**	**α6 WT**	**α6 (-/-)**
Saccharin	100 ± 10.1	100 ± 8.24	100 ± 14.7	41 ± 6.7***
Nicotine	58 ± 6.2**	104 ± 12.2	46 ± 7.3***	41 ± 8.5***
Mecamylamine	46 ± 3.7***	51 ± 12**	Not done	Not done

Significance of difference from own WT saccharin-treated group:

** $p < 0.01$,

*** $p < 0.01$.