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Nicotinic receptors as CNS targets for Parkinson's disease

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Abstract

Parkinson's disease is a debilitating neurodegenerative movement disorder characterized by damage to the nigrostriatal dopaminergic system. Current therapies are symptomatic only and may be accompanied by serious side effects. There is therefore a continual search for novel compounds for the treatment of Parkinson's disease symptoms, as well as to reduce or halt disease progression. Nicotine administration has been reported to improve motor deficits that arise with nigrostriatal damage in parkinsonian animals and in Parkinson's disease. In addition, nicotine protects against nigrostriatal damage in experimental models, findings that have led to the suggestion that the reduced incidence of Parkinson's disease in smokers may be due to the nicotine in tobacco. Altogether, these observations suggest that nicotine treatment may be beneficial in Parkinson's disease. Nicotine interacts with multiple nicotinic receptor (nAChR) subtypes in the peripheral and central nervous system, as well as in skeletal muscle. Work to identify the subtypes affected in Parkinson's disease is therefore critical for the development of targeted therapies. Results show that striatal $\alpha 6\beta 2$ -containing nAChRs are particularly susceptible to nigrostriatal damage, with a decline in receptor levels that closely parallels losses in striatal dopamine. In contrast, $\alpha 4\beta 2$ -containing nAChRs are decreased to a much smaller extent under the same conditions. These observations suggest that development of nAChR agonists or antagonists targeted to $\alpha 6\beta 2$ -containing nAChRs may represent a particularly relevant target for Parkinson's disease therapeutics.

Keywords

α -ConotoxinMII; Nicotine; Nicotinic; Parkinson's disease; Nigrostriatal; Striatum

1. Parkinson's disease and the nicotinic cholinergic system

The pathological hallmarks of Parkinson's disease are the presence of intracellular Lewy bodies and an extensive degeneration of the nigrostriatal dopaminergic system. [1–4]. There is a $\geq 70\%$ decline in striatal dopamine and $\geq 50\%$ loss of nigral dopaminergic neurons with the onset of clinical symptoms, which include bradykinesia, rigidity, and tremor [1–4].

Although Parkinson's disease has primarily been considered a dopaminergic disorder, it is becoming increasingly clear that multiple CNS systems are involved in its pathogenesis [5–7]. Braak and coworkers have also identified Lewy bodies in numerous non-dopaminergic brain regions including the locus coeruleus, raphe nuclei, thalamus, amygdala, olfactory nuclei, pedunclopontine nucleus, and cerebral cortex [5,6]. These observations are in agreement with much earlier studies, which indicated that multiple CNS neuronal systems are affected in

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^{*} indicates the possible presence of other subunits in the receptor complex

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Parkinson's disease [8,9]. Significant declines have been observed in molecular markers of many neurotransmitters and neuromodulators, such as enkephalin, somatostatin, cholecystokinin, and substance P, as well as changes in the noradrenergic, glutamatergic, serotonergic, and cholinergic systems [8,9].

This review focuses on the involvement of the nicotinic cholinergic system in Parkinson's disease for the following reasons. First, its close association and functional interaction with the nigrostriatal dopaminergic system suggests that nicotine or nicotinic agonists have potential in treatment of the motor symptoms of the disease [10,11]. In addition, the finding that stimulation of the nicotinic cholinergic system ameliorates cognitive declines [12] suggests that nicotinic acetylcholine receptor (nAChR) drugs may be useful in treating some of the nondopaminergic deficits associated with Parkinson's disease, such as dementia that develops in 50% of Parkinson's disease patients with disease progression [1–4]. Lastly, there is very compelling epidemiological evidence demonstrating a negative association between smoking and Parkinson's disease that has been attributed to the nicotine in tobacco, at least in part [11,13–17].

Altogether these observations suggest that the nicotinic cholinergic system represents a promising direction for much-needed therapies for Parkinson's disease. Moreover, the identification of unique nAChR subtypes in the nigrostriatal system (as discussed later) may allow for the development of drugs that selectively target this debilitating neurological disorder.

2. Nicotine and nicotinic agonists for neuroprotection

A wealth of epidemiological studies has demonstrated an inverse correlation between smoking and development of Parkinson's disease. The decreased incidence of Parkinson's disease in smokers has consistently been demonstrated in both prospective and retrospective studies, is dose- and time-dependent, and does not appear to be due to increased mortality [11,13–17]. Although the component in cigarette smoke that confers this apparent neuroprotective action remains to be identified, numerous studies using both culture systems and experimental animal models suggest that nicotine may play an important role, as detailed below.

2.1. Nicotine-mediated protection against toxic insults in culture models

Extensive evidence using culture systems has shown that nicotine protects against toxicity mediated by β -amyloid, glutamate, ethanol, trophic factor deprivation, arachidonic acid and others [11,18–20]. Of more direct relevance are studies using cultured ventral mesencephalic dopaminergic neurons which show that nicotine pre-treatment attenuates toxicity-induced by MPP⁺, the metabolite of MPTP that selectively destroys dopaminergic terminals [21]. There is thus a firm basis for the hypothesis that nicotine has neuroprotective properties using *in vitro* systems.

2.2. Nicotine-mediated protection against toxic insults in experimental animal models

There is also a large body of literature demonstrating nicotine-mediated protection against dopaminergic damage in parkinsonian animal models [22]. In rats, nicotine pre-treatment protects against nigrostriatal degeneration induced by the dopaminergic neurotoxin 6-hydroxydopamine or by mechanical lesions [23–28]. A neuroprotective effect of nicotine is also observed in striatum of mice in which nigrostriatal damage is induced by the selective dopaminergic neurotoxin MPTP [24,29–39]. It should be noted, however, that results are somewhat inconsistent in this model with protection in some but not all studies. Although the nature of this variability is uncertain, it may be due to the relatively large lesion generated with MPTP. This possibility stems from findings that neuroprotection is observed in mice treated

with methamphetamine and paraquat, both of which result in milder damage to the nigrostriatal dopaminergic system [24,40].

Nicotine-induced protection against striatal damage is also observed in MPTP-treated nonhuman primates, a model that may more closely resemble the human disease [41–43]. A chronic nicotine treatment regimen improved a variety of neurochemical markers in MPTP-lesioned nicotine treated animals, including tyrosine hydroxylase, the dopamine and vesicular monoamine transporters, dopamine levels and nAChR expression [42,43]. In addition, nicotine treatment normalized lesion-induced overactivity of the nigrostriatal pathway and preserved synaptic plasticity lost with nigrostriatal damage [41].

These combined results from different experimental animal models support the idea that the reduced incidence of Parkinson's disease in smokers may relate to the nicotine in tobacco.

3. Nicotine and nicotinic agonists for the symptomatic treatment of Parkinson's disease

Results from animal studies also lend support to the idea that nicotine and/or nAChR agonists may prove useful for treatment of Parkinson's disease symptoms. NACHR stimulation is well known to modulate locomotor activity in unlesioned animals [44], as well as ameliorate motor behaviors in animals with nigrostriatal damage. In rats with a unilateral 6-hydroxydopamine lesion, nicotine or nicotinic agonist treatment reduces apomorphine-induced turning behavior [45,46]. Treatment with nicotine or the nicotinic agonist SIB-1508Y also improves the antiparkinsonian action of levodopa in MPTP-lesioned monkeys [47,48]. Interestingly, nAChR activation also ameliorated deficits associated with nigrostriatal damage in tasks that evaluated aspects of attentional and executive cognitive functions [49].

The effect of smoking, and nicotine or nicotinic agonist treatment on Parkinson's disease symptoms has also been investigated (Table 1). Smoking (possibly acting via nicotine) reduced the tremor, rigidity, bradykinesia and gait disturbances observed in Parkinson's disease, although effects were transient and short lasting [50]. In another report, improvement was noted in two elderly patients with Parkinson's disease treated with the nicotine gum and patch for several months [51]. Variable results have been obtained in more recent small-scale clinical trials. Kelton and co-workers [52] observed improvements in areas of cognitive performance and motor measures after treatment with intravenous nicotine and the nicotine patch, with effects sustained for up to 1 month after the drug. However, Vieregge et al. observed no improvements in patients administered the patch for 3 weeks [53], although the lack of efficacy may have related to the fact that Parkinsonism was only assessed 3 weeks after patch cessation. On the other hand, Lemay et al. [54] also obtained no improvement in motor or cognitive deficits with immediate testing of Parkinson's disease symptoms after 3 to 4 weeks of treatment. Several studies also investigated the effect of acute exposure (1 day) to the nicotine patch or gum, with improvement in one but a worsening of motor performance in Parkinson's disease patients in two other studies [55,56]. There was also no observable effect of the nicotinic agonist SIB-1508Y on parkinsonism after 4 weeks of treatment [57]. Possible reasons for these varying effects on Parkinson's disease symptoms among the different studies may include differences in the severity of parkinsonism, the end-points under study, the study design (open-label versus blinded) and/or the dose, timing and mode of administration of nicotine.

Similarly variable results have been obtained in nonhuman primates. In our results in nonhuman primates, nicotine administration via the drinking water did not modify Parkinsonism over an 8-week period [58], although Domino et al. [47] did obtain small effects on motor activity using a different short term injection treatment protocol. On the other hand, although nicotine treatment had no effect in the short term in our monkey studies, longer term administration via

the drinking water (12 months) did improve striatal dopaminergic measures decreased with nigrostriatal damage [41–43]. These latter findings may suggest that nicotine treatment primarily has a protective and/or restorative effect with chronic use, but no acute symptomatic effects on Parkinsonism, at least at the doses tested to date.

4. Striatal cholinergic system

In order to understand how nAChR activation is involved in neuroprotection and/or symptomatic improvements in Parkinson's disease, knowledge of the anatomical relationship between the striatal cholinergic and dopaminergic systems is critical. Two major neuronal cell types are present in the striatum. These include the GABAergic medium spiny projection neurons that comprise the greater majority (~95%) of neurons, as well as a much smaller population of interneurons (5%) of which the majority are GABAergic and about a third cholinergic. These larger cholinergic interneurons form a dense network in striatum that closely overlaps with dopaminergic terminals [10]. Thus high levels of acetylcholine, choline acetyltransferase and acetylcholinesterase [59] coincide with expression of dopamine, tyrosine hydroxylase and other dopaminergic markers [60]. Under physiological conditions, the cholinergic interneurons are tonically active [10]. They release acetylcholine, which subsequently interacts with nAChRs on striatal cell bodies and nerve terminals, including dopaminergic projections from the substantia nigra and glutamatergic afferents from the cortex [61–64]. Stimulation of nAChRs at these sites results in dopamine release mediated through various nAChR subtypes [65–67], as discussed in the following section.

5. Nicotinic receptors in the nigrostriatal system

Identification of the nAChRs that modulate dopaminergic function and characterization of changes in their expression with nigrostriatal damage is essential for the development of Parkinson's disease therapies using nAChR ligands. NACHRs are pentameric ligand gated ion channels comprised of different combinations of α and β subunits to form heteromeric receptors, and select α subunits to form homomeric receptors [67–71]. There is a requirement for α subunits in the receptor complex, as these possess the recognition site for acetylcholine. The β subunit does not bind acetylcholine, however it modulates the interaction of the ligand with the α subunit, thereby affecting the physiological and pharmacological properties of the receptor [67–71]. Multiple nAChR subtypes (Fig. 1) have been identified in the nigrostriatal system using a variety of complementary experimental approaches.

5.1. Rodents

Robust nAChR expression has been identified in the rodent nigrostriatal system using radiolabeled epibatidine, a ligand that labels multiple receptor subtypes. The use of other ligands such as radiolabeled nicotine, α -bungarotoxin and α -conotoxinMII has helped identify nAChR subtypes, including those containing the $\alpha 4$, $\alpha 6$ and $\alpha 7$ subunits [67,69,71]. These findings from receptor binding studies are in agreement with the results of *in situ* hybridization and RT-PCR work, which demonstrate the presence of $\alpha 2$ through $\alpha 7$ and $\beta 2$ through $\beta 4$ nAChR subunit transcripts in the striatum and/or substantia nigra [72–77]. The results of immunoprecipitation studies using subunit-selective nAChR antibodies suggest that the greater majority of these transcripts are translated into protein to form receptors [64,78]. These show that all but the $\alpha 2$, $\alpha 3$ and $\beta 4$ subunits are present in the striatum and/or substantia nigra of mice and rats. These data, coupled with the results of functional studies (^3H -dopamine release from striatal synaptosomes) using wild-type and nAChR null mutant mice, indicate that the major nAChR subtypes in rodent striatum are $\alpha 6\alpha 4\beta 2\beta 3$, $\alpha 6\beta 2\beta 3$ and $\alpha 4\beta 2^*$ (*indicates the possible presence of other subunits in the receptor complex) nAChRs (Fig. 1) [63,64,79,80]. In contrast, $\alpha 7$ nAChRs are present at a much lower density, particularly in rats [81].

5.2. Monkeys

Multiple nAChRs have also been identified in striatum of nonhuman primates. nAChR subunit mRNAs in the primate nigrostriatal system include the $\alpha 2$, $\alpha 4$ through $\alpha 7$, and $\beta 2$ through $\beta 4$ transcripts [82,83]. Receptor binding studies with ^{125}I -epibatidine demonstrate that receptors are expressed in both the striatum and substantia nigra [84–86]. Subsequent studies with more selective ligands, such as ^{125}I - α -conotoxinMII indicated the presence of $\alpha 6\beta 2^*$ nAChR subtypes, in the nigrostriatal system [84,87], while the use of ^{125}I -epibatidine in the presence of unlabelled α -conotoxinMII suggested the presence of $\alpha 4\beta 2^*$ receptors. Immunoprecipitation studies with receptor subunit-directed antibodies show that all subunits except $\alpha 5$ and $\beta 4$ are expressed in the primate striatum and substantia nigra. The major nAChR subtypes appear similar to those in the rodent and include $\alpha 6\alpha 4\beta 2\beta 3$, $\alpha 6\beta 2\beta 3$ and $\alpha 4\beta 2$ (Fig. 1), with only very low $\alpha 7$ nAChR expression [87,88]. There are some variations in the minor subtypes, with expression of $\alpha 4\alpha 2\beta 2$ and $\alpha 3\beta 2^*$ nAChRs in nonhuman primates, but not rodent striatum [87].

5.3. Humans

Although studies in human striatum are more limited, the nAChR subtypes appear to be similar to those in the rodent and monkey nigrostriatal systems [89,90]. Evidence for this stems from the results of *in situ* hybridization and RT-PCR studies which demonstrate the presence of multiple nAChR transcripts including $\alpha 3$, $\alpha 4$, $\alpha 7$, and $\beta 2$ mRNAs, with the others not yet investigated [91–94]. Radioligand binding studies show that ^{125}I -epibatidine, ^3H -nicotine, ^{125}I -A85380, ^{125}I - α -bungarotoxin and ^{125}I - α -conotoxinMII all bind to human striatum suggesting the presence of nAChRs containing the $\alpha 4$, $\alpha 3$ and/or $\alpha 6$, $\alpha 7$, and $\beta 2$ [89, 90,95–104]. The $\alpha 4$, $\alpha 6$, $\beta 2$ and $\beta 3$ nAChR subunits have also been identified in human striatum using immunoprecipitation studies with subunit specific human nAChR antibodies [105]. These combined data suggest the presence of $\alpha 4\beta 2^*$, $\alpha 6\beta 2^*$ and $\alpha 7$ nAChRs in human striatum (Fig. 1).

6. Alterations in striatal nAChRs with nigrostriatal damage

A key question is whether nAChRs are altered with nigrostriatal damage in experimental models and in Parkinson's disease. Such knowledge is critical for the development of effective targeted therapies in Parkinson's disease. To approach this, work has been done in animal models with nigrostriatal damage, as well as with tissue from Parkinson's disease brains.

6.1. Rodents

The most commonly used parkinsonian rat model involves unilateral 6-hydroxydopamine injection into the striatum, substantia nigra or medial forebrain bundle. Nigrostriatal damage decreases high affinity nicotine binding sites that are thought to represent $\alpha 4\beta 2^*$ nAChRs, while $\alpha 7$ nAChRs are unaffected [106,107]. Nigrostriatal damage also leads to dramatic declines in binding of ^{125}I - α -conotoxinMII [108], a ligand that identifies $\alpha 6\beta 2^*$ in rodent striatum [80,109]. The loss of $\alpha 6\beta 2^*$ receptors correlates well with declines in the striatal dopamine transporter, a dopaminergic neuronal marker [64,78,108], suggesting that this receptor subtype is located primarily on striatal dopaminergic nerve terminals. The results of immunoprecipitation studies using nAChR subunit-targeted antibodies support these findings and show that the largest reductions are in $\alpha 6$ and $\beta 3$ subunit proteins, followed by smaller declines in $\alpha 4$, $\alpha 5$ and $\beta 2$ subunits [64,78]. There was no change in $\alpha 7$ nAChRs, while the $\alpha 2$, $\alpha 3$ and $\beta 4$ subunit proteins are not expressed in rodent striatum.

These combined results indicate that there is a loss of both $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs with nigrostriatal damage in rodents. The larger decrease in the latter population most likely occurs

because $\alpha 6\beta 2^*$ nAChRs are localized primarily on striatal dopaminergic terminals, while $\alpha 4\beta 2^*$ receptors are expressed on both dopaminergic terminals and other striatal neurons.

6.2. Monkeys

These data in rodents correspond well with those in nonhuman primates with MPTP-induced nigrostriatal damage (Table 2). A moderate lesion led to declines predominately in $\alpha 6\beta 2^*$ nAChRs that closely paralleled reductions in the dopamine transporter [84]. With severe nigrostriatal damage ($\geq 90\%$), decreases were also evident in $\alpha 4\beta 2^*$ nAChR although they were of smaller magnitude compared to those in $\alpha 6\beta 2^*$ nAChRs [85,110]. Immunoprecipitation studies with antibodies to human nAChR subunits showed that the major reductions with severe nigrostriatal damage were in the $\alpha 6$ and $\beta 3$ subunits, followed by decreases in $\alpha 4$, $\beta 2$, and $\alpha 3$ subunits [87]. There was no change in $\alpha 7$ nAChRs, while the $\alpha 5$ and $\beta 4$ subunit proteins are not expressed in monkey striatum [87].

In summary, $\alpha 6\beta 2^*$ nAChRs appear particularly vulnerable to nigrostriatal damage as they are selectively decreased with a moderate lesion. In contrast, $\alpha 4\beta 2^*$ subtypes are affected only with severe degeneration, while $\alpha 7$ nAChRs are unchanged (Table 2).

6.3. Humans

Results in brains of Parkinson's disease cases were similar to those in parkinsonian animal models. Declines were observed in radiolabeled epibatidine, which targets most nAChR subtypes and ^{125}I -A85380, which identifies $\beta 2$ -containing nAChRs [89,90,111]. Receptor studies with ^3H -nicotine also demonstrate $\sim 50\%$ decline in striatum, as well as in other areas including substantia nigra, cortex and hippocampus [98–101]. These latter findings support the contention that there is a generalized loss of neuronal integrity in Parkinson's disease [5, 6]. As in the rodent and monkey studies, declines in $\alpha 6\beta 2^*$ nAChRs in Parkinson's disease brains are significantly greater than in $\alpha 4\beta 2^*$ receptors in some striatal regions, as evaluated using receptor binding assays [103,104]. This observation is supported by results from immunoprecipitation studies which showed that the $\alpha 6$ subunit is decreased by $\geq 80\%$, whereas the $\alpha 4$ and $\beta 2$ subunits were decreased by $\sim 50\%$ [105].

Thus, both $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs were decreased in Parkinson's disease brains, with somewhat greater declines in this latter receptor population. There appeared to be little change in the $\alpha 7$ nAChR population, although declines have been observed in some studies [90,111].

7. Receptor studies with the α -conotoxinMII analog E11A

7.1. Identification of multiple $\alpha 6\beta 2^*$ nAChR subtypes in control striatum across species

As already mentioned, the $\alpha 6\beta 2^*$ nAChR population may be of particular relevance to nigrostriatal function and to Parkinson's disease [71]. This idea is based on results showing that its localization is fairly restricted in the CNS to the dopaminergic nigrostriatal and a few other catecholaminergic systems. In addition, declines in $\alpha 6\beta 2^*$ nAChRs closely parallel the loss of the striatal dopamine transporter. Previous rodents studies have shown that mouse striatum expresses two $\alpha 6\beta 2^*$ nAChR subtypes, those containing the $\alpha 6\alpha 4\beta 2\beta 3$ and $\alpha 6\beta 2\beta 3$ subunits [63,79]. Similarly, our studies in monkeys suggest the presence of at least two striatal $\alpha 6\beta 2^*$ nAChRs [87,112]. In a continued effort to more precisely determine the subunit composition of these receptors, experiments were recently performed with α -conotoxinMII analogs to determine whether these might discriminate between $\alpha 6\beta 2^*$ subtypes. One such analog α -conotoxinMII E11A, in which the glutamic acid at position 11 is replaced with alanine [113], inhibited ^{125}I - α -conotoxinMII binding to striatum from mice, monkeys and humans in a biphasic manner (Fig. 2) [88]. These data indicate that α -conotoxinMII E11A distinguishes between a very high (fM) and high affinity (pM) $\alpha 6\beta 2^*$ subtype. Subsequent receptor studies

showed that only the very high affinity site was lost in striatum of $\alpha 4$ null mutant mice (Fig. 2) [88]. Altogether, these data suggest that the very high affinity site is the $\alpha 6\alpha 4\beta 2\beta 3$ receptor and the high affinity site the $\alpha 6\beta 2\beta 3$ subtype.

7.2. Preferential loss of the $\alpha 6\alpha 4\beta 2\beta 3$ nAChR subtype with nigrostriatal damage in mice, monkeys and humans

We subsequently investigated the effect of nigrostriatal lesioning on these two striatal $\alpha 6\beta 2^*$ populations in two animal models and in Parkinson's disease (Fig. 2). There was a preferential loss of the very high affinity E11A binding site ($\alpha 6\alpha 4\beta 2\beta 3$) in striatum of MPTP-treated mice, MPTP-treated monkeys and Parkinson's disease cases [88]. This selective loss of the $\alpha 6\alpha 4\beta 2\beta 3$ binding site correlated well with reductions in striatal dopamine transporter [88]. In monkeys with moderate nigrostriatal damage (~50% reduction in dopamine transporter) there was a preferential reduction in the $\alpha 6\alpha 4\beta 2\beta 3$ subtype (Fig. 2 and 3), while the $\alpha 6\beta 2\beta 3$ and $\alpha 4\beta 2$ nAChR populations were reduced primarily with severe lesions ($\geq 90\%$) (Fig. 3; Table 2) [88]. The $\alpha 6\alpha 4\beta 2\beta 3$ subtype may thus provide a unique marker for dopamine nerve terminals that are particularly sensitive to nigrostriatal degeneration. This possibility is consistent with previous work indicating that dopaminergic afferents from the nigra to the striatum are variably susceptible to nigrostriatal insults [114,115]. Studies to understand the molecular basis for this enhanced vulnerability have linked calbindin to nigrostriatal dopamine neuron survival [116–118] while neuromelanin has been negatively associated [119–122]. However, a definitive connection of either of these markers with nigrostriatal damage remains to be established. On the other hand, there was a fairly clear-cut relationship between the loss of the $\alpha 6\alpha 4\beta 2\beta 3$ nAChR and nigrostriatal damage, possibly indicating that this subtype identifies selectively vulnerable dopaminergic terminals. Studies to investigate this possibility may also provide insight about the mechanisms responsible for the nigrostriatal neurodegeneration.

8. Concluding remarks

Accumulating evidence suggests that nigrostriatal nAChRs represent potential targets for the treatment of Parkinson's disease for symptomatic improvements and/or for long-term neuroprotection. Several nAChR populations have been identified in the nigrostriatal system. These include $\alpha 4\beta 2^*$ and $\alpha 6\beta 2\beta 3$ subtypes that are decreased primarily with severe nigrostriatal degeneration, and the $\alpha 6\alpha 4\beta 2\beta 3$ subtype that is significantly reduced even with only moderate damage (Fig. 3). These latter findings suggest that the $\alpha 6\alpha 4\beta 2\beta 3$ nAChR subtype represents a marker for neurons particularly vulnerable to nigrostriatal damage. In addition, these data suggest that this receptor subtype may represent a particularly relevant target for Parkinson's disease therapeutics as stimulation of the remaining $\alpha 6\alpha 4\beta 2\beta 3$ nAChRs may enhance function lost with nigrostriatal damage. This possibility stems from results showing that chronic nicotine treatment improved striatal integrity and function. On the other hand, nicotine is well known to desensitize or block nAChRs raising the question whether nicotine may induce its beneficial effects by blocking aberrant nAChR-mediated activity. This latter interpretation would suggest that an $\alpha 6\alpha 4\beta 2\beta 3$ nAChR antagonist (and not an agonist) may be the drug of choice to counteract the effects of nigrostriatal injury and/or provide symptomatic improvements. Continued studies to elucidate the role of $\alpha 6\beta 2^*$ nAChRs in nigrostriatal function should help resolve what type of $\alpha 6\beta 2^*$ nAChR ligand would prove most beneficial against the effects of nigrostriatal damage in neurological disorders such as Parkinson's disease.

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References

1. Samii A, Nutt JG, Ransom BR. Parkinson's disease. *Lancet* 2004;363:1783–93. [PubMed: 15172778]

2. Olanow CW. The scientific basis for the current treatment of Parkinson's disease. *Annu Rev Med* 2004;55:41–60. [PubMed: 14746509]
3. Savitt JM, Dawson VL, Dawson TM. Diagnosis and treatment of Parkinson disease: molecules to medicine. *J Clin Invest* 2006;116:1744–54. [PubMed: 16823471]
4. Lang AE, Obeso JA. Challenges in Parkinson's disease: restoration of the nigrostriatal dopamine system is not enough. *Lancet Neurol* 2004;3:309–16. [PubMed: 15099546]
5. Braak H, Del Tredici K, Bratzke H, Hamm-Clement J, Sandmann-Keil D, Rub U. Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages). *J Neurol* 2002;249(Suppl 3):III/1–5.
6. Braak H, Muller CM, Rub U, Ackermann H, Bratzke H, de Vos RA, Del Tredici K. Pathology associated with sporadic Parkinson's disease--where does it end? *J Neural Transm* 2006;(Suppl):89–97.
7. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 2003;24:197–211. [PubMed: 12498954]
8. Marsden CD. Basal ganglia disease. *Lancet* 1982;2:1141–7. [PubMed: 6128460]
9. Agid Y, Javoy-Agid F. Peptides and Parkinson's disease. *Trends in Neuroscience* 1985;6:30–35.
10. Zhou FM, Wilson CJ, Dani JA. Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J Neurobiol* 2002;53:590–605. [PubMed: 12436423]
11. Quik M. Smoking, nicotine and Parkinson's disease. *Trends Neurosci* 2004;27:561–8. [PubMed: 15331239]
12. Levin ED, McClernon FJ, Rezvani AH. Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. *Psychopharmacology (Berl)* 2006;184:523–39. [PubMed: 16220335]
13. Morens DM, Grandinetti A, Reed D, White LR, Ross GW. Cigarette smoking and protection from Parkinson's disease: false association or etiologic clue? *Neurology* 1995;45:1041–51. [PubMed: 7783862]
14. Baron JA. Beneficial effects of nicotine and cigarette smoking: the real, the possible and the spurious. *Br Med Bull* 1996;52:58–73. [PubMed: 8746297]
15. Checkoway H, Nelson LM. Epidemiologic approaches to the study of Parkinson's disease etiology. *Epidemiology* 1999;10:327–36. [PubMed: 10230846]
16. Gorell JM, Rybicki BA, Johnson CC, Peterson EL. Smoking and Parkinson's disease: a dose-response relationship. *Neurology* 1999;52:115–9. [PubMed: 9921857]
17. Allam MF, Campbell MJ, Hofman A, Del Castillo AS, Fernandez-Crehuet Navajas R. Smoking and Parkinson's disease: systematic review of prospective studies. *Mov Disord* 2004;19:614–21. [PubMed: 15197698]
18. O'Neill MJ, Murray TK, Lakics V, Visanji NP, Duty S. The role of neuronal nicotinic acetylcholine receptors in acute and chronic neurodegeneration. *Curr Drug Target CNS Neurol Disord* 2002;1:399–411.
19. Mudo G, Belluardo N, Fuxe K. Nicotinic receptor agonists as neuroprotective/neurotrophic drugs. Progress in molecular mechanisms. *J Neural Transm* 2007;114:135–47. [PubMed: 16906354]
20. Zanardi A, Leo G, Biagini G, Zoli M. Nicotine and neurodegeneration in ageing. *Toxicol Lett* 2002;127:207–15. [PubMed: 12052660]
21. Jeyarasasingam G, Tompkins L, Quik M. Stimulation of non- $\alpha 7$ nicotinic receptors partially protects dopaminergic neurons from 1-methyl-4-phenylpyridinium-induced toxicity in culture. *Neuroscience* 2002;109:275–85. [PubMed: 11801364]
22. Quik M, O'Neill M, Perez XA. Nicotine neuroprotection against nigrostriatal damage: importance of the animal model. *Trends Pharmacol Sci.* 2007
23. Janson AM, Fuxe K, Agnati LF, Kitayama I, Harfstrand A, Andersson K, Goldstein M. Chronic nicotine treatment counteracts the disappearance of tyrosine-hydroxylase-immunoreactive nerve cell bodies, dendrites and terminals in the mesostriatal dopamine system of the male rat after partial hemitransection. *Brain Res* 1988;455:332–45. [PubMed: 2900058]

24. Ryan RE, Ross SA, Drago J, Loiacono RE. Dose-related neuroprotective effects of chronic nicotine in 6-hydroxydopamine treated rats, and loss of neuroprotection in $\alpha 4$ nicotinic receptor subunit knockout mice. *Br J Pharmacol* 2001;132:1650–6. [PubMed: 11309235]
25. Soto-Otero R, Mendez-Alvarez E, Hermida-Ameijeiras A, Lopez-Real AM, Labandeira-Garcia JL. Effects of (-)-nicotine and (-)-cotinine on 6-hydroxydopamine-induced oxidative stress and neurotoxicity: relevance for Parkinson's disease. *Biochem Pharmacol* 2002;64:125–35. [PubMed: 12106613]
26. Abin-Carriquiry JA, McGregor-Armas R, Costa G, Urbanavicius J, Dajas F. Presynaptic involvement in the nicotine prevention of the dopamine loss provoked by 6-OHDA administration in the substantia nigra. *Neurotox Res* 2002;4:133–9. [PubMed: 12829413]
27. Costa G, Abin-Carriquiry JA, Dajas F. Nicotine prevents striatal dopamine loss produced by 6-hydroxydopamine lesion in the substantia nigra. *Brain Res* 2001;888:336–342. [PubMed: 11150495]
28. Visanji NP, O'Neill MJ, Duty S. Nicotine, but neither the $\alpha 4\beta 2$ ligand RJR2403 nor an $\alpha 7$ nAChR subtype selective agonist, protects against a partial 6-hydroxydopamine lesion of the rat median forebrain bundle. *Neuropharmacology* 2006;51:506–16. [PubMed: 16814329]
29. Gao ZG, Cui WY, Zhang HT, Liu CG. Effects of nicotine on 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine-induced depression of striatal dopamine content and spontaneous locomotor activity in C57 black mice. *Pharmacol Res* 1998;38:101–6. [PubMed: 9721596]
30. Janson AM, Fuxe K, Goldstein M. Differential effects of acute and chronic nicotine treatment on MPTP-(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced degeneration of nigrostriatal dopamine neurons in the black mouse. *Clin Investig* 1992;70:232–8.
31. Shahi GS, Das NP, Mochhala SM. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity: partial protection against striato-nigral dopamine depletion in C57BL/6J mice by cigarette smoke exposure and by β -naphthoflavone-pretreatment. *Neurosci Lett* 1991;127:247–50. [PubMed: 1881637]
32. Janson AM, Fuxe K, Agnati L, Sundstrom E, Goldstein M. The effect of chronic nicotine treatment on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced degeneration of nigrostriatal dopamine neurons in the black mouse. *Advances in Pharmacological Sciences* 1991;1:323–329.
33. Behmand RA, Harik SI. Nicotine enhances 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity. *J Neurochem* 1992;58:776–9. [PubMed: 1445501]
34. Hadjiconstantinou M, Hubble JP, Wemlinger TA, Neff NH. Enhanced MPTP neurotoxicity after treatment with isoflurophate or cholinergic agonists. *J Pharmacol Exp Ther* 1994;270:639–44. [PubMed: 7915317]
35. Ferger B, Spratt C, Earl CD, Teismann P, Oertel WH, Kuschinsky K. Effects of nicotine on hydroxyl free radical formation in vitro and on MPTP-induced neurotoxicity in vivo. *Naunyn Schmiedebergs Arch Pharmacol* 1998;358:351–9. [PubMed: 9774223]
36. Parain K, Hapdey C, Rousselet E, Marchand V, Dumery B, Hirsch EC. Cigarette smoke and nicotine protect dopaminergic neurons against the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine Parkinsonian toxin. *Brain Res* 2003;984:224–32. [PubMed: 12932857]
37. Serksen H, Hashim A, Wiener HL, Lajtha A. Effect of chronic oral nicotine on dopaminergic function in the MPTP-treated mouse. *Neurosci Lett* 1988;93:270–4. [PubMed: 3266524]
38. Carr LA, Rowell PP. Attenuation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity by tobacco smoke. *Neuropharmacology* 1990;29:311–4. [PubMed: 2325834]
39. Perry TL, Hansen S, Jones K. Exposure to cigarette smoke does not decrease the neurotoxicity of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. *Neurosci Lett* 1987;74:217–20. [PubMed: 3494963]
40. Khwaja M, McCormack A, McIntosh JM, Di Monte DA, Quik M. Nicotine partially protects against paraquat-induced nigrostriatal damage in mice; link to $\alpha 6\beta 2^*$ nAChRs. *Journal of Neurochemistry* 2006;100:180–190. [PubMed: 17227438]
41. Quik M, Chen L, Parameswaran N, Xie X, Langston JW, McCallum SE. Chronic oral nicotine normalizes dopaminergic function and synaptic plasticity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned primates. *J Neurosci* 2006;26:4681–9. [PubMed: 16641249]

42. Bordia T, Parameswaran N, Fan H, Langston JW, McIntosh JM, Quik M. Partial recovery of striatal nicotinic receptors in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys with chronic oral nicotine. *J Pharmacol Exp Ther* 2006;319:285–92. [PubMed: 16837557]
43. Quik M, Parameswaran N, McCallum SE, Bordia T, Bao S, McCormack A, Kim A, Tyndale RF, Langston JW, Di Monte DA. Chronic oral nicotine treatment protects against striatal degeneration in MPTP-treated primates. *J Neurochem* 2006;98:1866–75. [PubMed: 16882311]
44. Balfour DJ, Fagerstrom KO. Pharmacology of nicotine and its therapeutic use in smoking cessation and neurodegenerative disorders. *Pharmacol Ther* 1996;2:51–81.
45. Meshul CK, Kamel D, Moore C, Kay TS, Krentz L. Nicotine alters striatal glutamate function and decreases the apomorphine-induced contralateral rotations in 6-OHDA-lesioned rats. *Exp Neurol* 2002;175:257–74. [PubMed: 12009777]
46. Cosford ND, Bleicher L, Herbaut A, McCallum JS, Vernier JM, Dawson H, Whitten JP, Adams P, Chavez-Noriega L, Correa LD, Crona JH, Mahaffy LS, Menzaghi F, Rao TS, Reid R, Saccaan AI, Santori E, Stauderman KA, Whelan K, Lloyd GK, McDonald IA. (S)-(-)-5-ethynyl-3-(1-methyl-2-pyrrolidinyl)pyridine maleate (SIB-1508Y): a novel anti-parkinsonian agent with selectivity for neuronal nicotinic acetylcholine receptors. *J Med Chem* 1996;39:3235–7. [PubMed: 8765504]
47. Domino EF, Ni L, Zhang H. Nicotine Alone and in Combination with l-DOPA Methyl Ester or the D(2) Agonist N-0923 in MPTP-Induced Chronic Hemiparkinsonian Monkeys. *Exp Neurol* 1999;158:414–421. [PubMed: 10415147]
48. Schneider JS, Pope-Coleman A, Van Velson M, Menzaghi F, Lloyd GK. Effects of SIB-1508Y, a novel neuronal nicotinic acetylcholine receptor agonist, on motor behavior in parkinsonian monkeys. *Mov Disord* 1998;13:637–42. [PubMed: 9686767]
49. Decamp E, Schneider JS. Effects of nicotinic therapies on attention and executive functions in chronic low-dose MPTP-treated monkeys. *Eur J Neurosci* 2006;24:2098–104. [PubMed: 17067307]
50. Ishikawa A, Miyatake T. Effects of smoking in patients with early-onset Parkinson's disease. *J Neurol Sci* 1993;117:28–32. [PubMed: 8410063]
51. Fagerstrom KO, Pomerleau O, Giordani B, Stelson F. Nicotine may relieve symptoms of Parkinson's disease. *Psychopharmacology (Berl)* 1994;116:117–9. [PubMed: 7862924]
52. Kelton MC, Kahn HJ, Conrath CL, Newhouse PA. The effects of nicotine on Parkinson's disease. *Brain Cogn* 2000;43:274–82. [PubMed: 10857708]
53. Vieregge A, Sieberer M, Jacobs H, Hagenah JM, Vieregge P. Transdermal nicotine in PD: a randomized, double-blind, placebo-controlled study. *Neurology* 2001;57:1032–5. [PubMed: 11571330]
54. Lemay S, Chouinard S, Blanchet P, Masson H, Soland V, Beuter A, Bedard MA. Lack of efficacy of a nicotine transdermal treatment on motor and cognitive deficits in Parkinson's disease. *Prog Neuropsychopharmacol Biol Psychiatry* 2004;28:31–9. [PubMed: 14687854]
55. Ebersbach G, Stock M, Muller J, Wenning G, Wissel J, Poewe W. Worsening of motor performance in patients with Parkinson's disease following transdermal nicotine administration. *Mov Disord* 1999;14:1011–3. [PubMed: 10584678]
56. Clemens P, Baron JA, Coffey D, Reeves A. The short-term effect of nicotine chewing gum in patients with Parkinson's disease. *Psychopharmacology (Berl)* 1995;117:253–6. [PubMed: 7753975]
57. Shoulson I. Randomized placebo-controlled study of the nicotinic agonist SIB-1508Y in Parkinson disease. *Neurology* 2006;66:408–10. [PubMed: 16476941]
58. Parameswaran N, Cox H, O'Leary K, Langston JW, Di Monte D, Quik M. Nicotine treatment reduces L-dopa-induced dyskinesias in MPTP-treated squirrel monkeys. *Soc Neurosci Abstr*. 2007
59. Fonnum F. Recent developments in biochemical investigations of cholinergic transmission. *Brain Res* 1973;62:497–507. [PubMed: 4128242]
60. Fuxe K, Hoekfelt T, Nilsson O. Observations on the Cellular Localization of Dopamine in the Caudate Nucleus of the Rat. *Z Zellforsch Mikrosk Anat* 1964;63:701–6. [PubMed: 14254762]
61. 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;58:312–8. [PubMed: 10908298]

62. Marchi M, Risso F, Viola C, Cavazzani P, Raiteri M. Direct evidence that release-stimulating $\alpha 7^*$ nicotinic cholinergic receptors are localized on human and rat brain glutamatergic axon terminals. *J Neurochem* 2002;80:1071–8. [PubMed: 11953457]
63. Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC, Grady SR. Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Mol Pharmacol* 2004;65:1526–35. [PubMed: 15155845]
64. Champiaux N, Gotti C, Cordero-Erausquin M, David DJ, Przybylski C, Lena C, Clementi F, Moretti M, Rossi FM, Le Novere N, McIntosh JM, Gardier AM, Changeux JP. Subunit composition of functional nicotinic receptors in dopaminergic neurons investigated with knock-out mice. *J Neurosci* 2003;23:7820–9. [PubMed: 12944511]
65. Wonnacott S. Presynaptic nicotinic ACh receptors. *Trends Neurosci* 1997;20:92–8. [PubMed: 9023878]
66. MacDermott AB, Role LW, Siegelbaum SA. Presynaptic ionotropic receptors and the control of transmitter release. *Annu Rev Neurosci* 1999;22:443–85. [PubMed: 10202545]
67. Dani JA, Bertrand D. Nicotinic Acetylcholine Receptors and Nicotinic Cholinergic Mechanisms of the Central Nervous System. *Annu Rev Pharmacol Toxicol* 2007;47:699–729. [PubMed: 17009926]
68. Gotti C, Riganti L, Vailati S, Clementi F. Brain neuronal nicotinic receptors as new targets for drug discovery. *Curr Pharm Des* 2006;12:407–28. [PubMed: 16472136]
69. Gotti C, Zoli M, Clementi F. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol Sci* 2006;27:482–91. [PubMed: 16876883]
70. Nashmi R, Lester HA. CNS localization of neuronal nicotinic receptors. *J Mol Neurosci* 2006;30:181–4. [PubMed: 17192671]
71. Quik M, McIntosh JM. Striatal $\alpha 6^*$ nicotinic acetylcholine receptors: potential targets for Parkinson's disease therapy. *J Pharmacol Exp Ther* 2006;316:481–9. [PubMed: 16210393]
72. Wada E, Wada K, Boulter J, Deneris E, Heinemann S, Patrick J, Swanson LW. Distribution of $\alpha 2$, $\alpha 3$, $\alpha 4$, and $\beta 2$ neuronal nicotinic receptor subunit mRNAs in the central nervous system: a hybridization histochemical study in the rat. *J Comp Neurol* 1989;284:314–35. [PubMed: 2754038]
73. Le Novere N, Zoli M, Changeux JP. Neuronal nicotinic receptor $\alpha 6$ subunit mRNA is selectively concentrated in catecholaminergic nuclei of the rat brain. *Eur J Neurosci* 1996;8:2428–39. [PubMed: 8950106]
74. Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP. Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. *J Neurosci* 2001;21:1452–63. [PubMed: 11222635]
75. Charpantier E, Barneoud P, Moser P, Besnard F, Sgard F. Nicotinic acetylcholine subunit mRNA expression in dopaminergic neurons of the rat substantia nigra and ventral tegmental area. *Neuroreport* 1998;9:3097–101. [PubMed: 9804323]
76. Elliott KJ, Jones JM, Sacaan AI, Lloyd GK, Corey-Naeve J. 6-hydroxydopamine lesion of rat nigrostriatal dopaminergic neurons differentially affects nicotinic acetylcholine receptor subunit mRNA expression. *J Mol Neurosci* 1998;10:251–60. [PubMed: 9770646]
77. Winzer-Serhan UH, Leslie FM. Codistribution of nicotinic acetylcholine receptor subunit $\alpha 3$ and $\beta 4$ mRNAs during rat brain development. *J Comp Neurol* 1997;386:540–54. [PubMed: 9378850]
78. Zoli M, Moretti M, Zanardi A, McIntosh JM, Clementi F, Gotti C. Identification of the nicotinic receptor subtypes expressed on dopaminergic terminals in the rat striatum. *J Neurosci* 2002;22:8785–9. [PubMed: 12388584]
79. Luetje CW. Getting past the asterisk: the subunit composition of presynaptic nicotinic receptors that modulate striatal dopamine release. *Mol Pharmacol* 2004;65:1333–5. [PubMed: 15155826]
80. Whiteaker P, Peterson CG, Xu W, McIntosh JM, Paylor R, Beaudet AL, Collins AC, Marks MJ. Involvement of the $\alpha 3$ subunit in central nicotinic binding populations. *J Neurosci* 2002;22:2522–9. [PubMed: 11923417]
81. Clarke PB, Schwartz RD, Paul SM, Pert CB, Pert A. Nicotinic binding in rat brain: autoradiographic comparison of [3 H]acetylcholine, [3 H]nicotine, and [125 I]- α -bungarotoxin. *J Neurosci* 1985;5:1307–15. [PubMed: 3998824]

82. Han ZY, Le Novere N, Zoli M, Hill JA Jr, Champtiaux N, Changeux JP. Localization of nAChR subunit mRNAs in the brain of *Macaca mulatta*. *Eur J Neurosci* 2000;12:3664–74. [PubMed: 11029636]
83. Quik M, Polonskaya Y, Gillespie A, Jakowec M, Lloyd GK, Langston JW. Localization of nicotinic receptor subunit mRNAs in monkey brain by in situ hybridization. *J Comp Neurol* 2000;425:58–69. [PubMed: 10940942]
84. Quik M, Polonskaya Y, Kulak JM, McIntosh JM. Vulnerability of ^{125}I - α -conotoxin MII binding sites to nigrostriatal damage in monkey. *J Neurosci* 2001;21:5494–500. [PubMed: 11466420]
85. Kulak JM, McIntosh JM, Quik M. Loss of nicotinic receptors in monkey striatum after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment is due to a decline in α -conotoxin MII sites. *Mol Pharmacol* 2002;61:230–8. [PubMed: 11752225]
86. Han ZY, Zoli M, Cardona A, Bourgeois JP, Changeux JP, Le Novere N. Localization of [^3H]nicotine, [^3H]cytisine, [^3H]epibatidine, and [^{125}I] α -bungarotoxin binding sites in the brain of *Macaca mulatta*. *J Comp Neurol* 2003;461:49–60. [PubMed: 12722104]
87. Quik M, Vailati S, Bordia T, Kulak JM, Fan H, McIntosh JM, Clementi F, Gotti C. Subunit composition of nicotinic receptors in monkey striatum: effect of treatments with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or L-DOPA. *Mol Pharmacol* 2005;67:32–41. [PubMed: 15470079]
88. Bordia T, Grady SR, McIntosh JM, Quik M. Nigrostriatal damage preferentially decreases a subpopulation of $\alpha 6\beta 2^*$ nAChRs in mouse, monkey and Parkinson's disease striatum. *Mol Pharmacol*. 2007;10.1124/mol.107.035998
89. Court J, Clementi F. Distribution of nicotinic subtypes in human brain. *Alzheimer Dis Assoc Disord* 1995;9:6–14. [PubMed: 8534425]
90. Gotti C, Fornasari D, Clementi F. Human neuronal nicotinic receptors. *Prog Neurobiol* 1997;53:199–237. [PubMed: 9364611]
91. Hellstrom-Lindahl E, Mousavi M, Zhang X, Ravid R, Nordberg A. Regional distribution of nicotinic receptor subunit mRNAs in human brain: comparison between Alzheimer and normal brain. *Brain Res Mol Brain Res* 1999;66:94–103. [PubMed: 10095081]
92. Flora A, Schulz R, Benfante R, Battaglioli E, Terzano S, Clementi F, Fornasari D. Transcriptional regulation of the human $\alpha 5$ nicotinic receptor subunit gene in neuronal and non-neuronal tissues. *Eur J Pharmacol* 2000;393:85–95. [PubMed: 10771001]
93. Rubboli F, Court JA, Sala C, Morris C, Perry E, Clementi F. Distribution of neuronal nicotinic receptor subunits in human brain. *Neurochem Int* 1994;25:69–71. [PubMed: 7950973]
94. Guan ZZ, Nordberg A, Mousavi M, Rinne JO, Hellstrom-Lindahl E. Selective changes in the levels of nicotinic acetylcholine receptor protein and of corresponding mRNA species in the brains of patients with Parkinson's disease. *Brain Res* 2002;956:358–66. [PubMed: 12445706]
95. Whitehouse PJ, Martino AM, Wagster MV, Price DL, Mayeux R, Atack JR, Kellar KJ. Reductions in [^3H]nicotinic acetylcholine binding in Alzheimer's disease and Parkinson's disease: an autoradiographic study. *Neurology* 1988;38:720–3. [PubMed: 3362368]
96. Perry DC, Davila-Garcia MI, Stockmeier CA, Kellar KJ. Increased nicotinic receptors in brains from smokers: membrane binding and autoradiography studies. *J Pharmacol Exp Ther* 1999;289:1545–52. [PubMed: 10336551]
97. Court JA, Martin-Ruiz C, Graham A, Perry E. Nicotinic receptors in human brain: topography and pathology. *J Chem Neuroanat* 2000;20:281–98. [PubMed: 11207426]
98. Rinne JO, Myllykyla T, Lonnberg P, Marjamaki P. A postmortem study of brain nicotinic receptors in Parkinson's and Alzheimer's disease. *Brain Res* 1991;547:167–70. [PubMed: 1860068]
99. Lange KW, Wells FR, Jenner P, Marsden CD. Altered muscarinic and nicotinic receptor densities in cortical and subcortical brain regions in Parkinson's disease. *J Neurochem* 1993;60:197–203. [PubMed: 8417140]
100. Aubert I, Araujo DM, Cecyre D, Robitaille Y, Gauthier S, Quirion R. Comparative alterations of nicotinic and muscarinic binding sites in Alzheimer's and Parkinson's diseases. *J Neurochem* 1992;58:529–41. [PubMed: 1729398]
101. Perry EK, Morris CM, Court JA, Cheng A, Fairbairn AF, McKeith IG, Irving D, Brown A, Perry RH. Alteration in nicotine binding sites in Parkinson's disease, Lewy body dementia and

- Alzheimer's disease: possible index of early neuropathology. *Neuroscience* 1995;64:385–95. [PubMed: 7700528]
102. Sihver W, Gillberg PG, Nordberg A. Laminar distribution of nicotinic receptor subtypes in human cerebral cortex as determined by [³H](–)nicotine, [³H]cytisine and [³H]epibatidine in vitro autoradiography. *Neuroscience* 1998;85:1121–33. [PubMed: 9681951]
 103. Bohr IJ, Ray MA, McIntosh JM, Chalon S, Guilleau D, McKeith IG, Perry RH, Clementi F, Perry EK, Court JA, Piggott MA. Cholinergic nicotinic receptor involvement in movement disorders associated with Lewy body diseases. An autoradiography study using [¹²⁵I]α-conotoxinMII in the striatum and thalamus. *Exp Neurol* 2005;191:292–300. [PubMed: 15649484]
 104. Quik M, Bordia T, Forno L, McIntosh JM. Loss of α-conotoxinMII- and A85380-sensitive nicotinic receptors in Parkinson's disease striatum. *J Neurochem* 2004;88:668–79. [PubMed: 14720216]
 105. Gotti C, Moretti M, Bohr I, Ziabreva I, Vailati S, Longhi R, Riganti L, Gaimarri A, McKeith IG, Perry RH, Aarsland D, Larsen JP, Sher E, Beattie R, Clementi F, Court JA. Selective nicotinic acetylcholine receptor subunit deficits identified in Alzheimer's disease, Parkinson's disease and dementia with Lewy bodies by immunoprecipitation. *Neurobiol Dis* 2006;23:481–9. [PubMed: 16759874]
 106. Schwartz RD, Lehmann J, Kellar KJ. Presynaptic nicotinic cholinergic receptors labeled by [³H]acetylcholine on catecholamine and serotonin axons in brain. *J Neurochem* 1984;42:1495–8. [PubMed: 6707650]
 107. Clarke PB, Pert A. Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. *Brain Res* 1985;348:355–8. [PubMed: 4075093]
 108. Quik M, Sum JD, Whiteaker P, McCallum SE, Marks MJ, Musachio J, McIntosh JM, Collins AC, Grady SR. Differential declines in striatal nicotinic receptor subtype function after nigrostriatal damage in mice. *Mol Pharmacol* 2003;63:1169–79. [PubMed: 12695545]
 109. Champiaux N, Han ZY, Bessis A, Rossi FM, Zoli M, Marubio L, McIntosh JM, Changeux JP. Distribution and pharmacology of α6-containing nicotinic acetylcholine receptors analyzed with mutant mice. *J Neurosci* 2002;22:1208–17. [PubMed: 11850448]
 110. Kulak JM, Musachio JL, McIntosh JM, Quik M. Declines in different β2* nicotinic receptor populations in monkey striatum after nigrostriatal damage. *J Pharmacol Exp Ther* 2002;303:633–9. [PubMed: 12388645]
 111. Gotti C, Clementi F. Neuronal nicotinic receptors: from structure to pathology. *Prog Neurobiol* 2004;74:363–96. [PubMed: 15649582]
 112. Quik M, Bordia T, Okihara M, Fan H, Marks MJ, McIntosh JM, Whiteaker P. L-DOPA Treatment Modulates Nicotinic Receptors in Monkey Striatum. *Mol Pharmacol* 2003;64:619–28. [PubMed: 12920198]
 113. McIntosh JM, Azam L, Staheli S, Dowell C, Lindstrom JM, Kuryatov A, Garrett JE, Marks MJ, Whiteaker P. Analogs of α-conotoxin MII are selective for α6-containing nicotinic acetylcholine receptors. *Mol Pharmacol* 2004;65:944–52. [PubMed: 15044624]
 114. Gerfen CR, Herkenham M, Thibault J. The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. *J Neurosci* 1987;7:3915–34. [PubMed: 2891799]
 115. Langer LF, Graybiel AM. Distinct nigrostriatal projection systems innervate striosomes and matrix in the primate striatum. *Brain Res* 1989;498:344–50. [PubMed: 2477114]
 116. German DC, Manaye KF, Sonsalla PK, Brooks BA. Midbrain dopaminergic cell loss in Parkinson's disease and MPTP-induced parkinsonism: sparing of calbindin-D28k-containing cells. *Ann N Y Acad Sci* 1992;648:42–62. [PubMed: 1353337]
 117. Liang CL, Sinton CM, Sonsalla PK, German DC. Midbrain dopaminergic neurons in the mouse that contain calbindin-D28k exhibit reduced vulnerability to MPTP-induced neurodegeneration. *Neurodegeneration* 1996;5:313–8. [PubMed: 9117542]
 118. Gerfen CR, Baimbridge KG, Thibault J. The neostriatal mosaic: III. Biochemical and developmental dissociation of patch-matrix mesostriatal systems. *J Neurosci* 1987;7:3935–44. [PubMed: 2891800]
 119. Herrero MT, Hirsch EC, Kastner A, Ruberg M, Luquin MR, Laguna J, Javoy-Agid F, Obeso JA, Agid Y. Does neuromelanin contribute to the vulnerability of catecholaminergic neurons in monkeys intoxicated with MPTP? *Neuroscience* 1993;56:499–511. [PubMed: 8247275]

120. Hirsch E, Graybiel AM, Agid YA. Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. *Nature* 1988;334:345–8. [PubMed: 2899295]
121. McCormack AL, Di Monte DA, Delfani K, Irwin I, DeLanney LE, Langston WJ, Janson AM. Aging of the nigrostriatal system in the squirrel monkey. *J Comp Neurol* 2004;471:387–95. [PubMed: 15022260]
122. Zecca L, Zucca FA, Wilms H, Sulzer D. Neuromelanin of the substantia nigra: a neuronal black hole with protective and toxic characteristics. *Trends Neurosci* 2003;26:578–80. [PubMed: 14585596]

Abbreviations

nAChR
nicotinic acetylcholine receptor

Species	Subunit composition				References
Rodents Monkeys Humans					Klink et al., 2001, Whiteaker et al., 2002, Zoli et al., 2002, Azam et al., 2002, Cui et al., 2003, Salminen et al., 2004, Gotti et al., 2005, Quik et al., 2005, Gotti et al., 2006, Bordia et al., 2007
Rodents only					Zoli et al., 2002, Champiaux et al., 2003, Luesje 2004, Gotti et al., 2005
Monkeys only					Quik et al., 2005,

Fig 1.

Putative subunit composition of nAChR subtypes in rodent, monkey and human striatum. Note expression of the $\alpha 4 \beta 2$, $\alpha 6 \alpha 4 \beta 2 \beta 3$, $\alpha 6 \beta 2 \beta 3$ and $\alpha 7$ nAChR subtypes across the three species. In contrast, the $\alpha 4 \alpha 5 \beta 2$ and $\alpha 6(\text{non} \alpha 4) \beta 2^*$ nAChR subtypes appear to be expressed only in rodent striatum, while the $\alpha 4 \alpha 2 \beta 2$ and $\alpha 3 \beta 2^*$ subtypes are expressed in monkey striatum. The * indicates the possible presence of other nAChR subunits in the receptor complex.

Fig 2.

Preferential decline in the striatal $\alpha 6\alpha 4\beta 2\beta 3$ nAChR subtype in animal models (mouse and monkey) of nigrostriatal damage and Parkinson's disease. ^{125}I - α -conotoxinMII (0.5 nM) competition assays were performed using varying concentrations of the α -conotoxinMII analog E11A. In the upper left panel, biphasic inhibition curves (data fit best to a two site model) were obtained with E11A in the striatum of wild type mice suggesting the presence of at least two E11A-sensitive ^{125}I - α -conotoxinMII binding sites. In contrast monophasic inhibition (data fit best to a one site model) was obtained using striatal sections from $\alpha 4$ nAChR null mutant mice. Since these mice do not express the $\alpha 4$ subunit, the remaining high affinity-binding site is most likely the $\alpha 6\beta 2\beta 3$ nAChR, while the very high affinity-binding site that is lost may represent the $\alpha 6\alpha 4\beta 2\beta 3$ subtype. The three remaining panels depict the effect of nigrostriatal damage on the nAChR subtypes in mouse, monkey and human striatum. Biphasic inhibition curves (data fit best to two site model) were obtained with E11A under control conditions. These data suggest that E11A discriminates between at least two $\alpha 6\beta 2$ -containing nAChR populations, a very high $\alpha 6\alpha 4\beta 2\beta 3$ and a high affinity $\alpha 6\beta 2\beta 3$ nAChR subtype. Similar competition analyses using sections from lesioned animals and Parkinson's disease striatum yielded monophasic curves, suggesting the loss of the very high affinity E11A-sensitive $\alpha 6\alpha 4\beta 2\beta 3$ nAChR subtype. Symbols represent means $\pm$ S.E.M. of 4–8 mice, 4–6 monkeys and 5 control and 4 Parkinson's disease cases. Reproduced in modified form with permission from reference [88]

Fig 3.

Schematic representation of nAChR localization within the nigrostriatal pathway based on receptor binding, antibody immunoprecipitation and functional studies. The nigrostriatal dopaminergic terminals express three subtypes, the $\alpha 4\beta 2^*$, $\alpha 6\beta 2\beta 3$ and $\alpha 6\alpha 4\beta 2\beta 3$ nAChR populations. The results of lesion studies in animal models suggest that $\alpha 6\alpha 4\beta 2\beta 3$ subtype is localized to a select population of dopaminergic terminals that are particularly vulnerable to nigrostriatal insults (black "X"). The $\alpha 4\beta 2^*$ and $\alpha 6\beta 2\beta 3$ subtypes are most likely present on terminals other than those expressing $\alpha 6\alpha 4\beta 2\beta 3$ nAChRs since they are reduced only with more severe dopaminergic lesions (grey "X"). It is not known whether these latter two subtypes are on the same or separate dopaminergic terminals. The precise postsynaptic localization of the $\alpha 4\beta 2^*$ nAChR subtype in striatum is presently uncertain, but they may be present on cholinergic and/or GABAergic neurons. $\alpha 7$ nAChRs are located on glutamatergic afferent terminals from the cortex. ACh, acetylcholine; DA, dopamine; Glu, glutamate; SN, substantia nigra. The * indicates the possible presence of other nAChR subunits in the receptor complex.

Table 1
Summary of the studies testing nicotine or a nicotinic agonist in Parkinson's disease patients

Study	Test agent	Type of study	subjects [#]	Duration		Final dose/day	Anti-parkinsonian effect
				Dose titration	Dose maintenance		
Ishikawa & Miyatake, 1993	Smoking and nicotine gum	Open-label	6		Chronic smoker	NA	Yes
Fagerstrom et al., 1994	Nicotine gum and patch	Open-label	2		≥ 7 mo	15 mg patch + 3 × 2 mg gum	Yes
Clemens et al., 1995	Nicotine gum	Double-blinded placebo-controlled	48	ND	1 day	3 × 2 mg	No
Ebersbach et al., 1999	Nicotine patch	Double-blinded crossover	16	ND	12 hours	7 mg [#]	No
Kelton et al., 2000	Nicotine iv and patch	Open-label	15	2 wk	1 wk	14 mg	Yes
Vieregge et al., 2001	Nicotine patch	Double-blinded placebo-controlled	32	1 wk	2 wk	14 mg	No
Mitsuoka et al., 2002	Nicotine gum	Open-label	8	ND	1 day	NA	Yes
Lemav et al., 2004	Nicotine patch	Open-label	22	22 days	3 days	21 mg	No
Shoulson et al., 2006	SIB-1508Y	Double-blinded placebo controlled	77	2 wk	2 wk	10 mg	No

[#] Used the 35 mg patch, of which 14 mg is absorbed over 24 h, or 7 mg over 12 h. ND – not done; NA, not available.

Table 2

Summary of the declines in different nAChR subtypes in monkey striatum with nigrostriatal damage

nAChR subtype	Present on striatal dopamine terminals	Severity of lesion	
		Moderate	Severe
$\alpha 6\alpha 4\beta 2\beta 3$	Yes	↓↓	↓↓
$\alpha 6\beta 2\beta 3$	Yes	-	↓
$\alpha 4\beta 2$	Yes	-	↓
$\alpha 4\beta 2^*$	No	-	-
$\alpha 7$	No	-	-

The $\alpha 3\beta 2^*$ nAChR subtype is also expressed in monkey striatum and decreased with lesioning. However, the effect of moderate versus severe nigrostriatal damage remains to be determined [87]. The $\alpha 4\beta 2^*$ receptors may contain either $\alpha 4\beta 2$ or $\alpha 4\alpha 2\beta 2$ subunits. Moderate lesion, $\geq 50\%$ decline in the dopamine transporter; severe lesion, $\geq 90\%$ decline in the dopamine transporter; ↓ indicates a decline; - indicates no change.