



Smoking, nicotine and Parkinson's disease

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Epidemiological studies show that smoking is associated with a lower incidence of Parkinson's disease (PD). This finding is important because it could provide clues about therapeutic strategies for protection against this debilitating movement disorder. Smoke contains numerous chemicals that could be responsible for the apparent protective effect. Here, a role for nicotine is considered, because this chemical stimulates brain dopaminergic systems and provides some symptomatic benefit in PD. Nicotine also has a neuroprotective action. Putative factors and signaling pathways involved in the actions of nicotine are discussed. An understanding of the molecular basis for the reduced occurrence of PD in tobacco users is crucial for the development of intervention strategies to reduce or halt disease progression.

Parkinson's disease (PD) is a movement disorder occurring in ~1% of the population over the age of 55. It is characterized by selective damage to dopaminergic nigrostriatal neurons that leads to motor deficits including rigidity, tremor, bradykinesia and possibly dementia [1,2]. The etiology of PD is unknown, although the disease appears multifactorial in origin, possibly arising from a complex interaction between genetics and environment. The most commonly used treatment is dopamine replacement therapy. Although initially very effective, L-dopa provides only symptomatic relief with an inevitable disease progression. Moreover, it loses efficacy with time and is also plagued by the development of drug-induced side effects [1,2]. Interventions to delay, halt or even reverse the neurodegenerative process are therefore crucial. In fact, studies to identify relevant pharmacological compounds are currently in progress [3,4]. Candidate agents for testing exhibit a broad spectrum of biological actions and include nicotine.

The rationale for considering nicotine is based on epidemiological findings that PD is less prevalent in smokers. Indeed, this is one of the most robust observations linking environment and PD [5–8]. Although cigarette smoke contains thousands of chemicals, nicotine represents a possible candidate for two reasons. First, nicotine stimulates striatal dopamine neurons that are selectively damaged in PD. Second, nicotine exposure protects against neuronal insults in experimental models.

This inverse association between smoking and PD is consistently observed in >40 independent studies by different investigators conducted over the past 50 years. Furthermore, the effects of smoking are dose-dependent and related to number of cigarette-pack-years [5–8]. The finding that there is an inverse association in prospective studies argues against a selective mortality – that is, individuals prone to develop PD do not simply die early of smoking-related diseases [5–8]. One report showed that this decreased incidence is not observed with increased age, suggesting that smoking delays PD onset [9]. Although many questions remain, a recent study in twins demonstrating a reduced PD risk with smoking led the authors to conclude there is a 'true protective effect of cigarette smoking' [10].

The goal of this paper is to consider mechanisms that explain the apparent neuroprotective effect of smoking in PD, with a focus on nicotine for the reasons already mentioned. Because the classical action of nicotine is through stimulation of nicotinic ACh (nACh) receptors, the stage will be set by briefly describing the receptor subtypes in the nigrostriatal system and the changes with neuronal damage. This is important because such knowledge could allow the design of drugs that target the desired nACh receptor populations with a minimum of side effects. Next, the receptor-mediated and non-receptor-mediated actions of nicotine will be considered, along with the possibility that other substances in tobacco products play a contributory (or major?) role. Lastly, the basis for some of the conflicting results of smoking and nicotine exposure against nigrostriatal damage in animal models will be discussed. A critical examination of these data could provide clues about the biological processes that influence protection.

Presence of specific nACh receptor subtypes in the nigrostriatal system

Neuronal nACh receptors are pentameric ligand-gated ion channels composed of α subunits (homomeric receptors) or of α and β subunits (heteromeric receptors) [11]. Identification of receptor composition is proving challenging, with six different α subunits ($\alpha 2$ – $\alpha 7$) and three different β subunits ($\beta 2$ – $\beta 4$) expressed in mammalian brain [11]. mRNA encoding $\alpha 2$ – $\alpha 7$ and $\beta 2$ – $\beta 4$ subunits is present in the nigrostriatal system, with a very restricted localization of $\alpha 6$ and $\beta 3$ mRNA in the substantia nigra, the region of origin of striatal dopaminergic terminals [12,13]. Studies indicate that multiple receptor subtypes are localized in the striatum and substantia nigra, including $\alpha 4\beta 2^*$, $\alpha 6\beta 2^*$,

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Available online 25 June 2004

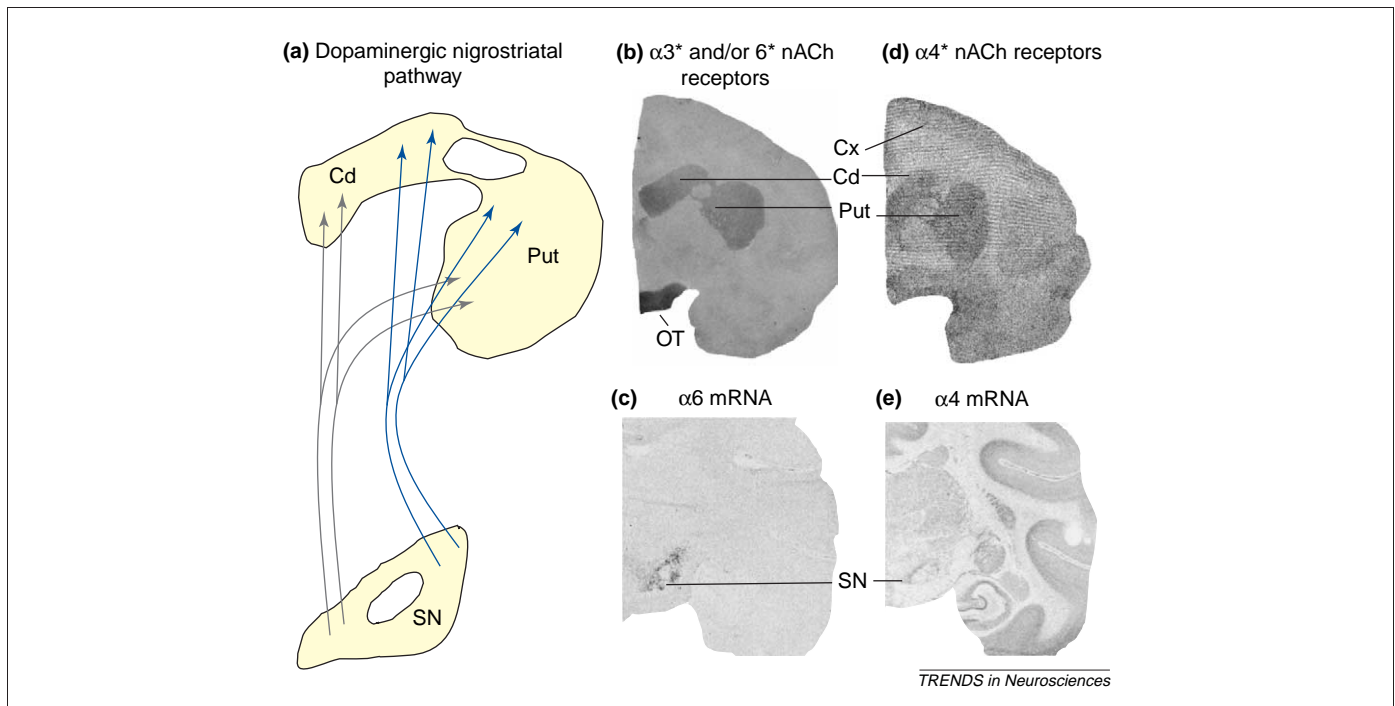


Figure 1. Autoradiographic distribution of nicotinic ACh (nACh) receptor mRNA and receptor sites at the level of the substantia nigra and striatum in monkey brain. (a) A representation of the nigrostriatal pathway. Dopaminergic neurons project from the substantia nigra (SN) to the caudate (Cd) and putamen (Put), together known as the striatum; the neuronal cell bodies in the medial portion of the substantia nigra tend to project to more medial and ventral areas of the caudate and putamen (gray pathway), whereas neurons in the lateral portion of the substantia nigra project to more lateral and ventral areas (violet pathway). Receptor-binding studies using ^{125}I -conotoxin-MII to detect $\alpha 3^*$ and/or $\alpha 6^*$ nACh receptors shows that there is select localization of $\alpha 3^*$ and/or $\alpha 6^*$ sites to the striatum, which comprises the caudate and putamen (b). $\alpha 3^*$ and/or $\alpha 6^*$ sites are also present in the optic tract (OT). In addition, note the restricted distribution of $\alpha 6$ mRNA to the substantia nigra (c), which contains the dopaminergic cell bodies that project to the striatum. By contrast, $\alpha 4^*$ nACh receptors, as measured using ^3H -cytisine (d), are much more widely distributed throughout the cortex (Cx) and other brain areas, as is $\alpha 4$ mRNA (e). Similar results are obtained in rodent brain with respect to the select distribution of $\alpha 3^*$ and/or $\alpha 6^*$ nACh receptor subtypes [14–20]. Part (b) reproduced, with permission, from Ref. [15] © (2001) by the Society for Neuroscience; (c,e) reproduced, with permission, from Ref. [12] © (2000) John Wiley and Sons, Inc.

$\alpha 4\alpha 6\beta 2^*$, $\alpha 4\alpha 5\beta 2^*$, $\beta 3^*$ and others [5,14–20] (asterisks indicate that other nACh receptor subunits can be present). Interestingly, $\alpha 6^*$ receptor subtypes are selectively localized to the nigrostriatal pathway and to only a limited number of other brain areas, suggesting they might be of particular relevance to striatal function (Figures 1 and 2).

Nigrostriatal damage reduces expression of specific nACh receptor subtypes

In PD there is a loss of nigrostriatal dopaminergic neurons. Thus, a crucial question is whether nACh receptors are affected by denervation, as this would have an impact on subsequent actions of administered nicotine. Results from animal models with selective lesion of nigrostriatal dopaminergic neurons and in PD (Figure 2 and Table 1) demonstrate significant declines in select nACh receptor populations [5,19–25]. In rodent and monkey striatum, both nicotine-binding sites (in $\alpha 4^*$ receptors) and ^{125}I - α -conotoxinMII sites (in $\alpha 3^*$ and/or $\alpha 6^*$ receptors) are decreased with lesioning, although there might be selective vulnerability of the ^{125}I - α -conotoxinMII sites in monkeys compared with rodents [5,15,19,20,23]. In humans with PD there are also reduced nicotine-binding sites and ^{125}I - α -conotoxinMII sites, as well as decreased $\alpha 3^*$ subunit immunoreactivity [21,24,25]. Striatal ^{125}I - α -bungarotoxin ($\alpha 7$) receptors have been difficult to detect in human, monkey and rat striatum using receptor autoradiography (Figure 2), although $\alpha 7$ nACh receptor

immunoreactivity has been identified in human striatum and shown not to be decreased with PD [24,25]. There are also measurable levels of ^{125}I - α -bungarotoxin sites in mouse brain, but no changes with nigrostriatal damage [23]. These findings suggest that $\alpha 7$ receptors are not present on striatal dopaminergic terminals (Figure 3). Overall, the combined results indicate that there are declines in $\alpha 2$ – $\alpha 6^*$, but not $\alpha 7^*$, nACh receptors with nigrostriatal damage across species. These data provide an experimental basis for the use of drugs targeted to $\alpha 2$ – $\alpha 6^*$ nACh receptors in PD.

Role of nACh receptors in modulating dopamine release

One important action of nicotine is modulation of dopamine release from nigrostriatal dopaminergic terminals [26–29]. The finding that nACh receptors are decreased with nigrostriatal damage suggests that nicotine-evoked dopamine release might also be reduced. Indeed, studies in mice [23] show that there is a decrease in nicotine-evoked dopamine release with nigrostriatal damage that parallels the nACh receptor decline (Figure 4). Drugs that target the subtypes of nACh receptor that decline with nigrostriatal damage might therefore be useful in treating PD, assuming that the remaining receptors are coupled to their effector mechanisms and not saturated with ACh. Nicotine itself might not be ideal because it interacts with numerous nACh receptor subtypes in both the central and the peripheral nervous systems, and it could result in harmful side effects and/or counteract positive actions at

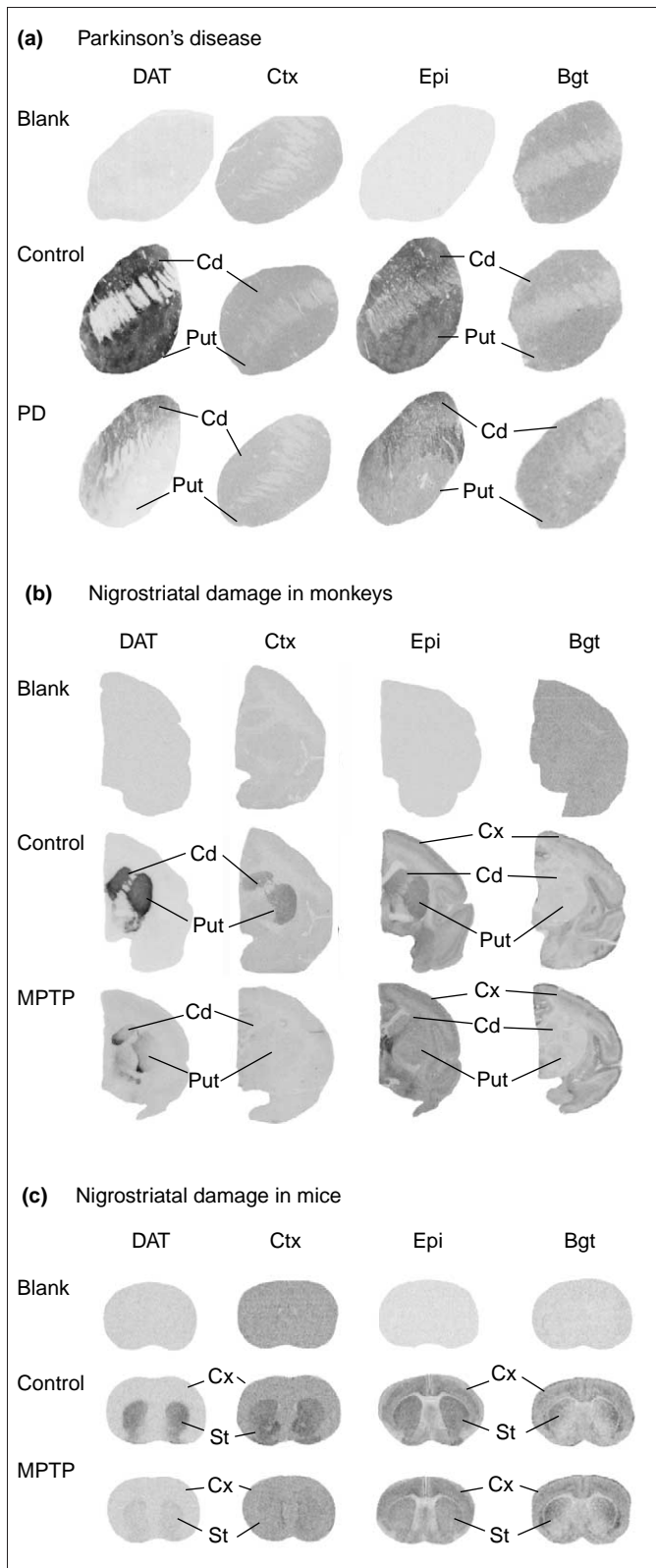


Figure 2. Differential changes in striatal nicotinic ACh (nACh) receptors with nigrostriatal damage. Computerized autoradiograms depicting the effect of nigrostriatal damage on the dopamine transporter (DAT) and different nACh receptor populations in human (a), monkey (b), and rodent (c) striatum. The dopamine transporter is depicted on the left, ^{125}I - α -conotoxinMII (Ctx; i.e. $\alpha 3^*$ and/or $\alpha 6^*$ nACh receptors) in the second column, ^{125}I -epibatidine (Epi; i.e. $\alpha 2^*$, $\alpha 3^*$, $\alpha 4^*$, $\alpha 5^*$ and $\alpha 6^*$ receptors) in the third column, and ^{125}I - α -bungarotoxin (Bgt; i.e. $\alpha 7^*$ nACh receptors) on the right. Expression of the dopamine transporter, a marker of dopaminergic terminals, is decreased by $\sim 90\%$ across species. ^{125}I - α -conotoxin binding is decreased in parallel with the dopamine transporter, with 90% fewer $\alpha 3^*$ and/or $\alpha 6^*$ sites following nigrostriatal damage in mouse and monkey striatum, and 50%

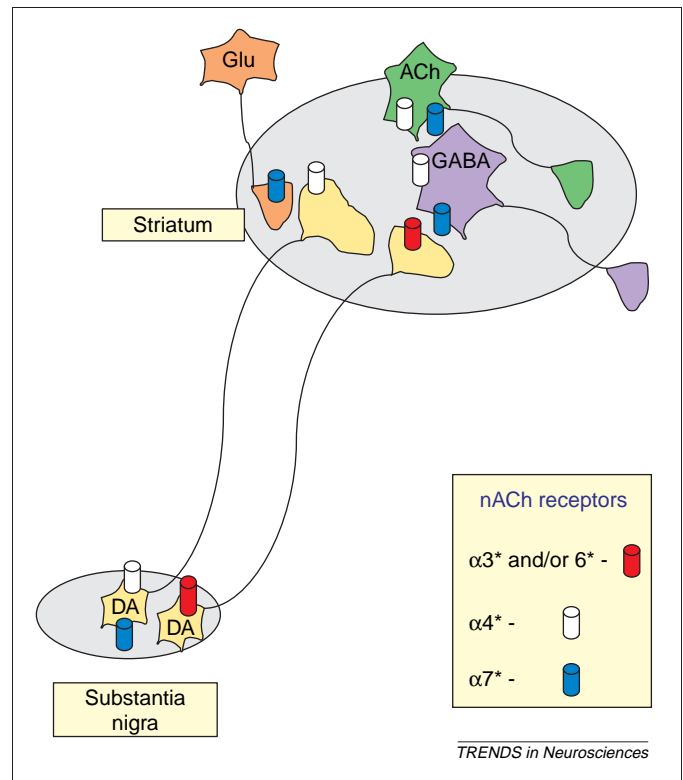


Figure 3. Nicotinic ACh (nACh) receptor localization in the nigrostriatal pathway. Combined results from receptor studies using radioligand binding, immunocytochemistry and immunoprecipitation suggest a differential localization of nACh receptor populations in striatum and substantia nigra. $\alpha 3^*$ and/or $\alpha 6^*$ subtypes appear to be localized primarily on dopaminergic (DA) neurons, whereas $\alpha 4^*$ receptors are distributed on both dopaminergic and nondopaminergic neurons. The latter could include striatal cholinergic interneurons and striatal medium spiny GABAergic neurons that project to the globus pallidus and substantia nigra. By contrast, $\alpha 7^*$ sites seem to be largely present on nondopaminergic pathways, including incoming glutamatergic (Glu) inputs from the cortex.

other sites. Drugs that target a subpopulation of nACh receptors could provide a superior effect. $\alpha 6^*$ nACh receptors might be particularly relevant because this subtype is the most sensitive to agonist-induced activation [30] (S. McCallum *et al.*, unpublished).

How nicotine-evoked dopamine release might benefit PD

The modulatory effect of nicotine on nigrostriatal dopamine release [26–29] is most likely of direct relevance to PD because there are major deficits in nigrostriatal function in this disorder. Conceivably, enhanced nicotine-evoked dopamine release could benefit PD (i) from an immediate symptomatic standpoint, by alleviating the

fewer ^{125}I - α -conotoxinMII sites in Parkinson's disease (PD) human striatum [22]. ^{125}I -Epibatidine sites are decreased by $\sim 50\%$ in all species with nigrostriatal damage. Striatal α -bungarotoxin receptors are barely detectable in monkey and human striatum (or in rat) using receptor autoradiography; they are present in mouse striatum but their presence is not altered with nigrostriatal damage. However, expression of $\alpha 7$ nACh receptors, as detected in human brain using $\alpha 7$ -subunit-selective antibodies, is not changed with nigrostriatal damage, in agreement with the mouse data [25]. Overall, the data across species are in agreement with respect to a decline in presence of $\alpha 2$ – $\alpha 6^*$ receptor subtypes following nigrostriatal damage. Abbreviations: Cd, caudate; Cx, cortex; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Put, putamen; St, striatum. Part (a) reproduced in part, with permission, from Ref. [22] © (2004) Blackwell Publishing; (b) MPTP DAT section and ^{125}I -epibatidine sections reproduced, with permission, from Ref. [66]; (c) reproduced in part, with permission, from Ref. [23].

Table 1. Consistent decline in number of non- $\alpha 7^*$ nACh receptors in rodent, monkey and human striatum with nigrostriatal damage^a

nACh receptor subtype	Receptor-binding studies	Antibody studies	Species	Change	Refs
$\alpha 2-\alpha 6^*$	^{125}I -epibatidine	$\alpha 4, \alpha 5, \alpha 6$	Rodent	↓	[19,20,23]
		–	Monkey	↓	[66]
		$\alpha 3^b$	Human	↓	[24,25]
$\alpha 3^*$ and/or $\alpha 6^*$	^{125}I - α -conotoxinMII	–	Rodent	↓	[23]
		–	Monkey	↓	[15]
		–	Human	↓	[22]
$\alpha 4^*$	^3H -nicotine, ^3H -cytisine, ^3H -ACh	–	Rodent	↓	[67,68]
		–	Monkey	↓ with severe lesion	^c
		–	Human	↓	[21,24]
$\alpha 7^*$	^{125}I - α -bungarotoxin	–	Rodent	None	[23]
		–	Monkey	None	^c
		$\alpha 7$	Human	None	[24,25]

^aNigrostriatal damage was induced by administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxydopamine to rodents or monkeys. Overall, the decreases in receptor number in Parkinson's disease brain parallel those in the animal models. Dashes indicate experiments not done; asterisks indicate that other nACh receptor subunits can be present. The antibody studies involve immunoprecipitation with $\alpha 2, \alpha 3, \alpha 4, \alpha 5, \alpha 6$ or $\alpha 7$ nicotinic ACh (nACh)-receptor-selective antibodies, with preferential declines in nACh receptors containing subunits recognized by the antibody indicated.

^bSome studies show no change in $\alpha 3$ nACh receptors using subtype-selective antibodies [24], although radiolabeled epibatidine binding is consistently decreased with nigrostriatal damage [24,25].

^cM. Quik *et al.*, unpublished.

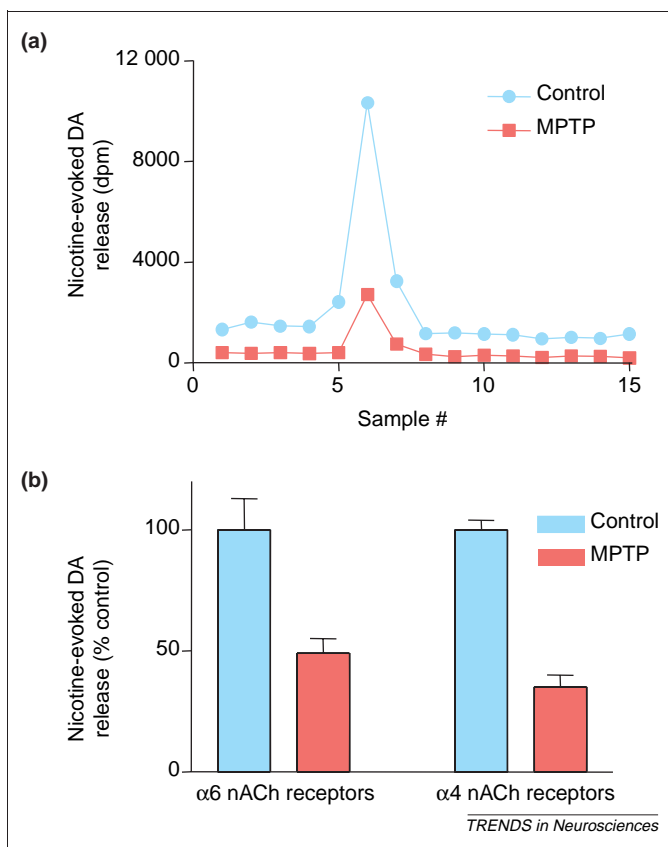


Figure 4. Reduction in nicotine-evoked dopamine (DA) release after nigrostriatal damage is closely coupled to denervation-induced declines in nicotinic ACh (nACh) receptors in mouse striatum. Studies investigated nicotine-evoked dopamine release from striatal synaptosomes prepared from striatum of mice that had been treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [23]. There is a significant reduction in nicotine-evoked dopamine release (measured in disintegrations per minute, dpm) after nigrostriatal damage (a); in mice, this reduction appears to be due to a decline in nicotine-evoked release mediated through both the $\alpha 4^*$ and the $\alpha 6^*$ nACh receptor populations [23] (b). This was determined by measuring nicotine-induced ^3H -dopamine release in both the absence and the presence of 30 nM α -conotoxinMII, a ligand that interacts with $\alpha 6^*$ nACh receptors in mouse brain. Thus, dopamine release in the presence of α -conotoxinMII occurs through stimulation of $\alpha 4^*$ nACh receptors. The $\alpha 6^*$ -nACh-receptor-mediated release component is the difference between total dopamine release and that occurring in the presence of α -conotoxinMII.

motor symptoms, and (ii) by protecting against nigrostriatal damage in the long term.

Symptomatic – nicotine as an adjunct therapy with L-dopa to relieve PD symptoms

Although L-dopa is one of the best treatments for PD, its use is limited because of the development of unwanted side effects. One approach to circumvent these difficulties is to reduce L-dopa dose; however, this strategy results in a worsening of parkinsonism [1,2]. Studies in nonhuman primates show that co-administration of a nicotinic agonist with a lower L-dopa dosage resulted in an improvement in parkinsonism similar to that seen with higher L-dopa dosage, although it led to a decline in motor complications such as dyskinesias [31]. These data suggest that combined treatment with nicotine, or preferably nicotinic agonists that selectively stimulate nACh receptor subtypes, could improve PD treatment.

Neuroprotective – nicotine reduces disease progression

In addition, enhanced nicotine-evoked dopamine release might also attenuate nigrostriatal damage and reduce PD progression. Converging evidence suggests that exposure to pesticides and/or toxins leads to nigrostriatal damage [32,33]. For instance, the active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that causes parkinsonism, is selectively transported into neurons via the same uptake system as dopamine [32]. Once inside the terminal, it interferes with mitochondrial function to result in nerve terminal degeneration. Nicotine therapy could reduce nigrostriatal damage by stimulating the release of dopamine that would then compete with toxin for entry into the nerve terminal. With less toxin in the terminal, less nerve cell damage might be expected [34]. In addition to this direct effect of nicotine on dopamine release, there are other potential mechanisms for nicotine-mediated neuroprotection, as will now be discussed.

Box 1. Discrepancies between Parkinson's disease (PD) and experimental models

Although smoking is associated with an undisputed reduction in PD, protection against neurotoxic insults in animals has yielded conflicting results [5,34,69]. This is not unexpected, for two reasons. First, the etiology of PD and the molecular basis for its progressive nature are presently unknown. This highlights the important issue of the limitations of animal models: they might not mirror the disease but simply model certain aspects, and must be interpreted with caution. Second, the agent(s) in smoke that protect against PD are uncertain, complicating interpretation of experimental studies that test single components. Possible reasons for some of the inconsistencies are outlined below.

(i) Animal models of PD

Neurodegenerative mechanisms, morphological changes and behavioral end-points will vary depending on the animal species (e.g. mouse, rat or monkey) and the nature of the nigrostriatal insult. For instance, the commonly used neurotoxins 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine and methamphetamine [70], and the more recently identified agents including rotenone and paraquat [32,71], all damage the nigrostriatal system but do so by somewhat different molecular mechanisms. In addition, transection of the nigrostriatal pathway can initiate distinct pathological processes.

(ii) Duration of the neurodegenerative process

PD is progressive in nature and occurs over many years [2]. However, the animal models involve acute or at best semi-chronic paradigms (e.g. occurring over weeks), primarily because of funding and time constraints [5,35,69]. Their relevance to PD warrants serious reflection. Chronic models could be crucial. Age is another important variable – most studies are conducted in young animals, whereas PD occurs with age. Uncontrolled environmental and genetic factors could also affect the neurodegenerative process in PD.

(iii) Treatment regimens

Drug dose and length of treatment (days to years) are significant variables. The treatment regimen (intermittent or constant) can also influence effectiveness of the neuroprotective agent. Nicotinic receptors desensitize rapidly and are upregulated with chronic dosing. Such regimens could, therefore, provide very different outcomes from those using intermittent therapy.

(iv) Inadequacy of a single drug regimen

A combination of tobacco components might be essential for mimicking effects of smoking against nigrostriatal damage [54,55,64,65].

The above points should all be considered when extrapolating animal data to PD. Then again, PD *per se* might be multifactorial in origin with the common end result being an enhanced susceptibility of melanized nigral neurons to a variety of different endogenous and exogenous insults. Thus, different animal models might be of relevance depending on the etiology of PD.

Other mechanisms whereby nicotine could protect against PD

Extensive literature suggests that nicotine protects against different toxic insults in culture systems [5,35], including against MPTP-induced toxicity in nigral neurons [36]. These *in vitro* results extend to the *in vivo* situation. Smoking and/or nicotine exposure protect against nigrostriatal damage in several rodent models. However, reproducibility is an issue (Box 1), with only some studies showing protection [5,35]. Identification of the biological bases for these inconsistencies is important because these provide insights into the mechanisms and factors that influence neuroprotection. Such knowledge

might also explain why only ~50% of smokers are protected against PD [5–8]. A discussion of the relevant issues is provided in Box 1.

Protection through receptor-mediated mechanisms

$\alpha 7^*$ nACh receptor stimulation is crucial for preventing glutamate, and other, toxicity throughout the brain [5,35]. However, evidence points to a role for non- $\alpha 7^*$ nACh receptors, possibly $\alpha 4^*$ subtypes, in nicotine-mediated protection against nigrostriatal injury [36–38]. Development of subtype-selective nicotinic ligands will help resolve this issue. Because the receptors flux Ca^{2+} and nACh-receptor-evoked function is Ca^{2+} -dependent, Ca^{2+} is likely to represent a first step in the intracellular signaling cascade [5,35,39,40]. Diverse downstream pathways and processes are subsequently activated (Figure 5), including the nitric oxide–cGMP pathway, caspases, apoptotic signaling and others [41–44]. Immune modulators, such as interleukin (IL)-1 α , IL-1 β , IL-6 and tumor necrosis factor α , are also triggered by nicotine exposure [45–47]. Activation of these signaling mechanisms might subsequently lead to neuroprotection through inhibition of toxin-induced apoptosis, and/or increased expression of neurotrophic factors crucial for neuronal maintenance, survival and regeneration [48,49].

Protection through non-receptor-mediated mechanisms

Although a major focus is on receptor-mediated protection, nicotine might also play a more direct role that bypasses nACh receptors. For instance, nicotine could enhance elimination [34] or suppress the formation of toxins by altering monoamine oxidase activity [50,51]. Nicotine might also act as an antioxidant [50,51] and/or inhibit complex I of the electron transport chain, with a consequent reduction in the levels of reactive oxygen species [52] (but see also Ref. [53]). Furthermore, nicotine could act by stimulating drug-metabolizing enzymes in the cytochrome P450 (CYP) family. CYP2E1, CYP2B6 and CYP2B1 are present in dopaminergic regions in the brain and are induced by nicotine at relatively low doses [54,55]. Enzyme activation can enhance the metabolism of toxic agents, thus lowering their levels and reducing neuronal damage. If nicotine functions through a non-receptor mechanism, nicotinic agonists with low potency, for example D-nicotine, could be very useful because they would exert a minimum of receptor-mediated side effects.

An alternative, or possibly complementary, hypothesis is that nicotine metabolites mediate effects presently attributed to nicotine. One particularly interesting compound is cotinine, a long-lived (~18 h) primary metabolite that exhibits cytoprotective properties in cultured cells through a non-receptor-mediated mechanism [56]. Another nicotine metabolite nornicotine reduced β -amyloid aggregation [57]. Conceivably, metabolites such as nornicotine could prevent aggregation of α -synuclein and reduce PD pathology, in analogy to their role in Alzheimer's disease [35].

Does nicotine therapy benefit PD?

The finding that smoking protects against PD raises the question whether nicotine treatment is beneficial either to

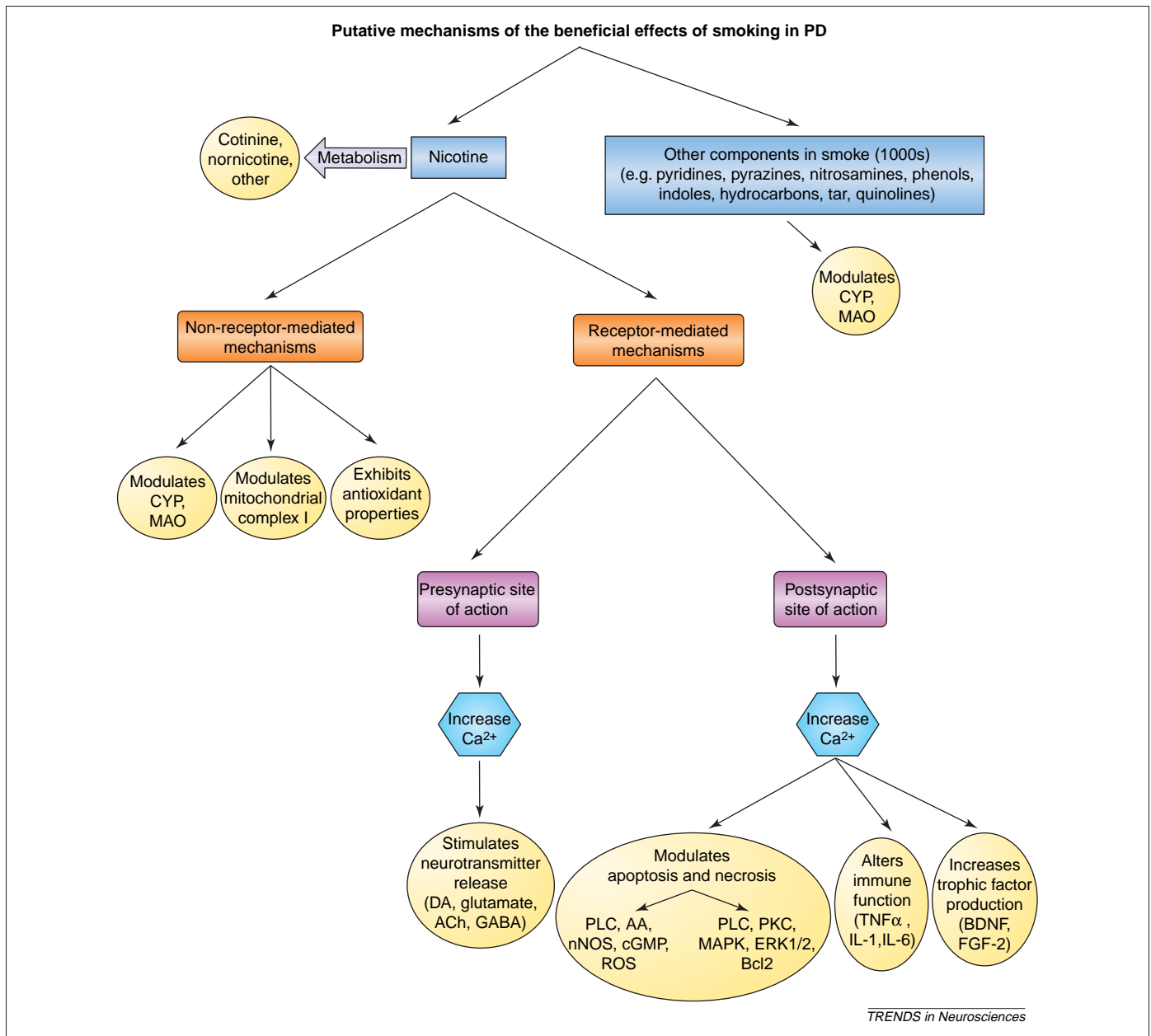


Figure 5. Potential mechanisms whereby smoking could result in an apparent beneficial effect in Parkinson's disease (PD). Smoke consists of >4000 chemicals that could conceivably affect PD. At present, there is a focus on nicotine because of its ability to stimulate dopamine (DA) release in the striatum and exert a neuroprotective action against toxic insults. Nicotine might produce these effects by directly influencing activities of enzymes such as monoamine oxidases (MAO) and members of the cytochrome P450 (CYP) family, by modulating mitochondrial complex I activity to preserve mitochondrial function and consequently reduce neuronal damage, or through a direct chemical action as an antioxidant. Nicotine could also act by stimulating nicotinic ACh (nACh) receptor sites. Numerous signaling pathways are altered in response to nACh receptor activation, including presynaptic pathways involved in control of neurotransmitter release and postsynaptic pathways involved in apoptosis and necrosis, immune modulation and trophic factor production. Metabolites of nicotine such as cotinine and nornicotine might also be beneficial. Abbreviations: AA, arachidonic acid; BDNF, brain derived neurotrophic factor; ERK, extracellular-signal-regulated protein kinase; FGF-2, fibroblast growth factor 2; IL, interleukin; MAPK, mitogen-activated protein kinase; nNOS, neuronal nitric oxide synthase; PKC, protein kinase C; PLC, phospholipase C; ROS, reactive oxygen species; TNF α , tumor necrosis factor α .

relieve PD symptoms or for neuroprotection. With regard to use of nicotine in symptomatic treatment, initial reports had suggested that smoking, nicotine patches or nicotine gum alleviate some movement disabilities [58,59]. More recently, several small-scale clinical trials tested the effect of short-term nicotine therapy on motor and cognitive deficits in PD. Overall, these studies generated inconsistent results [60–63]. One study [61] suggested a small improvement in cognitive and motor function; however, it was non-blind and effects were small. By contrast, Ebersbach *et al.* [62] showed a decline in motor

performance, and there was no effect in the two other studies [60,63]. These negative results might relate to the use of the nicotine patch which, unlike smoking, results in a constant release of nicotine that could induce chronic receptor desensitization. Lack of efficacy might also relate to the use of nicotine rather than subtype-directed nicotinic drugs that would target the receptors compromised in PD. The development of such nACh receptor drugs is actively being pursued [35] to combat memory deficits in Alzheimer's disease, although they have not yet been tested for PD. These drugs could also prove beneficial

Box 2. Key questions for future research

(i) What is the chemical agent that mediates the neuroprotective effects of smoking?

- Nicotine?
- Other compounds in smoke?
- Combinations of these?

(ii) If nicotine is involved, by what mechanism(s)?

This is an important point for the development of therapeutic strategies.

- By receptor-related mechanisms? (And if so, which receptor subtypes? Can they be targeted with drugs? Through what mechanism of action – trophic, anti-inflammatory or other?)
- Non-receptor-mediated mechanisms (e.g. enzyme induction, anti-oxidant properties or other?)
- Combinations of receptor-mediated and non-receptor-mediated mechanisms?

(iii) What is the relevance of current *in vivo* models to PD?

The animal model should mimic the human situation as closely as possible. The following points should therefore be carefully considered.

- Nature of the insult (i.e. the specific neurotoxin used for study)
- Time course of nigrostriatal damage (acute versus chronic)
- Nature of nicotine treatment (chronic versus intermittent)
- Species (e.g. rodents or monkeys)
- Gender
- Age of animals
- Genetic susceptibility

in treating cognitive deficits, a major problem in the later stages of PD.

To conclude, the therapeutic efficacy of nicotine as an adjunct therapy in the symptomatic treatment of PD requires further study, and its long-term neuroprotective potential has yet to be evaluated.

What about other chemicals in tobacco products?

In addition to nicotine, numerous agents in tobacco products could modulate biological functions and, thus, the development of PD. Cigarette smoking is associated with decreased (40%) brain monoamine oxidase B activity, an effect not mediated by nicotine (at least not acutely) [64]. This could contribute to a lower incidence of PD by decreasing levels of hydrogen peroxide, a by-product of dopamine metabolism, or by reducing enzymatic conversion of endogenous or exogenous compounds to toxic metabolites. Agents in tobacco products that inhibit monoamine oxidase B include 2,3,6-trimethyl-1,4-naphthoquinone, which also partially protects against neurotoxicity in mice [65]. Monoamine oxidase B inhibitors, such as selegiline and rasagiline, are also used in the symptomatic treatment of PD and could have neuroprotective potential [65]. Further research might identify other smoke components that attenuate PD pathology.

Concluding remarks and future challenges

A systematic review of the existing literature shows that there is an undoubted inverse correlation between smoking and PD; that is, disease incidence is unexpectedly reduced in smokers. The challenge at present is to identify the active component(s) in smoke that contributes to this beneficial effect. This is no easy task because exposure to the agent(s) of interest could occur many years before the

onset of symptoms. Some of the key issues for future research are outlined in Box 2. Although a role for nicotine is compelling, the involvement of other chemicals in cigarette smoke cannot be ruled out. Questions that should be asked next regard the mode(s) of action of nicotine and/or other smoke components, and putative signaling mechanisms (summarized in Figure 5). Resolution of these and other issues should provide the key to understanding the molecular basis for neuroprotection in PD with tobacco use. Such knowledge is important for the development of targeted drug therapies.

Acknowledgements

Research support is gratefully acknowledged from the TRDRP and NIH. Thanks to T. Bordia, A. Collins, B. Collier, D. Di Monte, A. Lai and S. McCallum for helpful comments and discussion.

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