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$\alpha 6\beta 2^*$ and $\alpha 4\beta 2^*$ Nicotinic Acetylcholine Receptors As Drug Targets for Parkinson's Disease

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Abstract—Parkinson's disease is a debilitating movement disorder characterized by a generalized dysfunction of the nervous system, with a particularly prominent decline in the nigrostriatal dopaminergic pathway. Although there is currently no cure, drugs targeting the dopaminergic system provide major symptomatic relief. As well, agents directed to other neurotransmitter systems are of therapeutic benefit. Such drugs may act by directly improving functional deficits

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in these other systems, or they may restore aberrant motor activity that arises as a result of a dopaminergic imbalance. Recent research attention has focused on a role for drugs targeting the nicotinic cholinergic systems. The rationale for such work stems from basic research findings that there is an extensive overlap in the organization and function of the nicotinic cholinergic and dopaminergic systems in the basal ganglia. In addition, nicotinic acetylcholine receptor (nAChR) drugs could have clinical potential for Parkinson's disease. Evidence for this proposition stems from studies with experimental animal models showing that nicotine pro-

TECTS AGAINST NEUROTOXIN-INDUCED NIGROSTRIATAL DAMAGE and improves motor complications associated with L-DOPA, the "gold standard" for Parkinson's disease treatment. Nicotine interacts with multiple central nervous system receptors to generate therapeutic responses but also produces side effects. It is important therefore to identify the nAChR subtypes most beneficial for treating Parkinson's disease. Here we review nAChRs with particular emphasis on the subtypes that contribute to basal ganglia function. Accumulating evidence suggests that drugs targeting $\alpha 6\beta 2^*$ and $\alpha 4\beta 2^*$ nAChR may prove useful in the management of Parkinson's disease.

I. Introduction—Parkinson's Disease and Links to the Nicotinic Cholinergic System

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease, and affects 2% of people over the age of 60 (Mayeux, 2003). It is a neurodegenerative movement disorder characterized by postural instability, bradykinesia and a generally asymmetric onset of tremor and rigidity (Lang, 2009; Poewe, 2009; Quik et al., 2009; Schapira, 2009; Feng and Maguire-Zeiss, 2010; Obeso et al., 2010). These motor symptoms are a consequence of degeneration of the nigrostriatal dopaminergic pathway, which is the most severely affected neurotransmitter system in Parkinson's disease. In addition, accumulating evidence shows that there is a generalized neuronal loss in the central and peripheral nervous system in this disorder (Braak et al., 2002, 2003). Numerous CNS¹ neurotransmitter systems degenerate, such as the adrenergic, cholinergic, serotonergic, glutamatergic, and GABAergic pathways, although to a lesser degree than the nigrostriatal dopaminergic pathway (Curzon, 1977; Haber, 1986; Dubois et al., 1990; Poewe, 2009). Damage to these other systems may contribute to the motor problems and also underlie the nonmotor symptoms associated with Parkinson's disease, including deficits in cognition/memory, affect, sleep/wakefulness, and autonomic function (Lang, 2009; Poewe, 2009; Quik et al., 2009; Schapira, 2009; Calabresi et al., 2010; Feng and Maguire-Zeiss, 2010; Obeso et al., 2010).

The etiology of Parkinson's disease is currently uncertain and has been attributed to a complex interplay between genetic and environmental factors (Schapira, 2009; Bekris et al., 2010; Obeso et al., 2010). A small minority of cases (~5%) is genetic (familial), with Mendelian inheri-

tance. Gene mutations linked to Parkinson's disease include *PARK1* to *PARK18*, which seem to be responsible for approximately 50% of familial cases and ~2% of sporadic forms (Schapira, 2009; Obeso et al., 2010). Of these, the most well studied include *PARK1/4*, which involves point mutations and multiplications in the α -synuclein gene. Deletions or point mutations in the *PARK2* gene, which encodes parkin, are linked to autosomal recessive juvenile-onset parkinsonism. Recessive mutations in *PARK6* or *PINK1* (which encodes a mitochondrial kinase) are responsible for a familial form of early-onset parkinsonism. Recessively inherited missense and exonic deletion mutations in *PARK7* or *DJ1* have also been reported although these are very rare. The most common mutations in either familial or sporadic Parkinson's disease involve mutations in *PARK8* or *LRRK2* (encoding leucine-rich repeat kinase 2). The LRRK2 protein contains both Rab GTPase and kinase enzymatic activities, which have been implicated in multiple neuronal functions under physiological conditions. In addition to genetic mutations, environmental factors have also been linked to the occurrence of Parkinson's disease. The greatest positive risk factor is pesticide exposure, whereas tobacco use has consistently been linked to a decreased incidence of Parkinson's disease (Quik et al., 2009).

The most effective current treatment for Parkinson's disease motor symptoms is dopamine replacement therapy with L-DOPA and/or dopamine agonists. These drugs are particularly beneficial for improving motor deficits in Parkinson's disease; however, side effects commonly arise and drug effectiveness diminishes with disease progression (Lang, 2009; Poewe, 2009; Quik et al., 2009; Schapira, 2009; Feng and Maguire-Zeiss, 2010; Obeso et al., 2010). Moreover, the nonmotor symptoms linked to Parkinson's disease, such as dementia, sleep deficits, depression, and others, are not improved with these pharmacotherapies. There is therefore a critical need to develop improved treatments for Parkinson's disease, ideally to halt disease progression but also to provide better symptomatic relief of the motor and nonmotor symptoms. The focus of this review is on a potential role for the nicotinic cholinergic system, based on the following rationale: a considerable literature demonstrates an extensive anatomical and functional overlap between the nicotinic cholinergic and dopa-

¹Abbreviations: 5-iodo-A-85380, 3-[(2S)-2-azetidinylmethoxy]-5-iodopyridine dihydrochloride; ABT-089, pozanicline; ACh, acetylcholine; AR-R17779, (2S)-2'H-spiro[4-azabicyclo[2.2.2]octane-2,5'-[1,3]oxazolidin]-2'-one; CNS, central nervous system; DARPP-32, 32-kDa dopamine- and cAMP-regulated phosphoprotein; GTS-21, 3-[(3E)-3-[(2,4-dimethoxyphenyl)methylidene]-5,6-dihydro-4H-pyridin-2-yl]pyridine; MPTP, 6-hydroxydopamine or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; nAChR, nicotinic acetylcholine receptor; NMDA, N-methyl-D-aspartate; P450, cytochrome P450; PNU-282987, N-(3R)-1-azabicyclo[2.2.2]oct-3-yl-4-chlorobenzamide; PP-1, protein phosphatase 1; PPT, pedunculopontine nucleus; SN, substantia nigra; SSR180711, (4-bromophenyl)-1,4-diazabicyclo[3.2.2]nonane-4-carboxylate; VTA, ventral tegmental area.

minergic systems in the nigrostriatal pathway, which plays a pivotal role in Parkinson's disease. In addition, accumulating studies suggest that drugs that interact at nAChRs, such as nicotine, may protect against nigrostriatal damage. Moreover, nicotine and nAChR drugs alleviate some of the motor side effects associated with dopamine replacement therapy. Finally, the emerging procognitive and antidepressant effects of nAChR drugs may offer therapeutic benefit for the dementia and depressive symptoms observed in Parkinson's disease.

II. Inter-Relationship between Nicotinic Cholinergic and Dopaminergic Systems

A. Striatum

The subcortical region, referred to as the striatum because of its striated or striped appearance, consists of the caudate nucleus and putamen. In rodents, the caudate and the putamen are merged, but in primates, these structures are separated by the internal capsule. The striatum is divided into dorsal and ventral territories. The dorsal striatum primarily receives dopaminergic innervation from the substantia nigra (SN) pars compacta, with little contribution from the ventral tegmental area (VTA) (Björklund and Dunnett, 2007) (Fig. 1). It is this nigrostriatal pathway that selectively degenerates in Parkinson's disease (Fig. 1) (Obeso et al., 2000; Parent et al., 2000; Smith and Kieval, 2000). Dopaminergic innervation of the ventral striatum (also known as the nucleus accumbens core) is from the VTA, with some input from the dorsal SN. Likewise, primate dorsal striatum primarily receives projections from the SN. However, there is a more pronounced "intermingling" of the pathways from the SN and VTA in the ventral striatum or nucleus accumbens in primates (Björklund and Dunnett, 2007). In Parkinson's disease, the dorsal striatum is affected to the greatest degree, reflecting the degeneration of the nigrostriatal pathway, whereas the mesolimbic projection from the VTA is relatively spared (Obeso et al., 2000; Parent et al., 2000; Smith and Kieval, 2000). Unless specified, the term "striatum" will henceforth be used to denote the striatal areas compromised in Parkinson's disease.

The striatum boasts some of the highest levels of dopamine and acetylcholine (ACh) in the brain (Fig. 2). Historically these two transmitters were viewed as having antagonistic roles (Calabresi et al., 2000; Cragg, 2006), reflecting the beneficial effects of muscarinic antagonists in Parkinson's disease (Langmead et al., 2008). The terminal fields of the dopaminergic afferents have extensive arborizations (Wilson and Groves, 1980). Thus, each dopaminergic afferent contacts a large area of the striatum to exert a coordinated influence (Fig. 2). The principal target is the GABAergic medium spiny projection neurons, which constitute more than 90% of the neuronal population in the striatum. These neurons form the direct and indirect output pathways to the basal ganglia whereby motor function is moderated (Bolam et al., 2000; Obeso et al., 2000; Parent

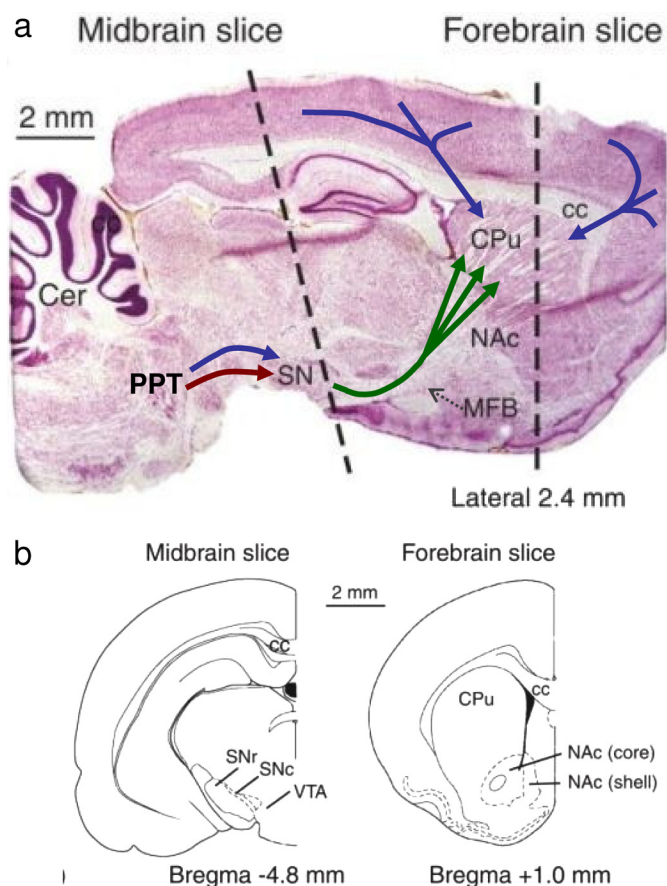


FIG. 1. Schematic representation of the nigrostriatal dopaminergic systems in the rat brain and its links to the pedunculo-pontine (PPT) nucleus. a, sagittal section, stained with cresyl violet, illustrating the nigrostriatal pathway (green) that projects from cell bodies in the SN pars compacta of the midbrain to the caudate-putamen (CPu; dorsal striatum) of the rat forebrain. Note its "striated" appearance. The ventral striatum, below the CPu, corresponds to the nucleus accumbens (NAc). The SN receives cholinergic (red) and glutamatergic (blue) inputs from the PPT in the brain stem. The CPu receives glutamatergic (blue) inputs from the somatosensory and association cortices. Cc, corpus callosum; Cer, cerebellum; MFB, medial forebrain bundle. b, transverse sections at the level of the dashed lines in a. Bregma coordinates indicate distance anterior (+) and posterior (-) to this landmark on the skull. [Reprinted from Rice ME, Avshalumov MV, and Patel JC (2007) Hydrogen peroxide as a diffusible messenger: evidence from voltammetric studies of dopamine release in brain slices, in *Electrochemical Methods for Neuroscience* (Michael AC and Borland LM eds) pp 205–232, CRC Press. Copyright © 2007 CRC Press. Used with permission.]

et al., 2000; Smith and Kieval, 2000). In the direct pathway, information from the striatum is transmitted directly to the output structures of the basal ganglia. These include the SN pars reticulata and the entopeduncular nucleus in rodents (the latter corresponds to the internal segment of globus pallidus in primates), and thence to the brainstem (for the control of head, neck, and eye movements involved in gaze and focus) or the thalamus and motor cortex, respectively (Fig. 3). The indirect pathway proceeds via the globus pallidus (the external segment of globus pallidus in primates) and subthalamic nucleus before reaching the SN pars reticulata and the entopeduncular nucleus. The dopaminergic input to the striatum from the SN represents a substantial feedback component of this circuitry (Bolam et al., 2000).

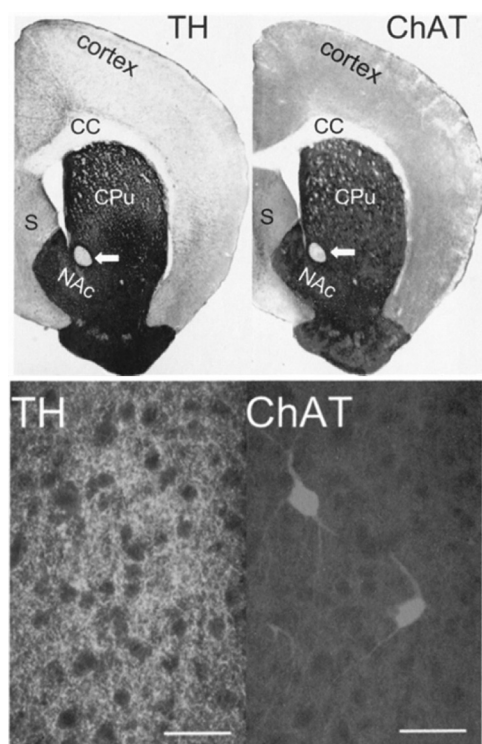


FIG. 2. Dense and overlapping distribution of ACh and dopamine in the rat striatum. Top, bright-field photomicrographs show tyrosine hydroxylase (TH) and choline acetyltransferase (ChAT) antibody staining of forebrain sections. Arrows, anterior commissure; CC, corpus callosum; CPu, caudate putamen; NAc, nucleus accumbens; S, septum. Bottom, higher magnification immunofluorescence images of striatum double labeled for TH (left) and ChAT (right), revealing sparse cholinergic interneurons and dense fiber tracts for both transmitters. Scale bars, 50 μm . [Reproduced from Zhou FM, Liang Y, and Dani JA (2001) Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nat Neurosci* 4:1224–1229. Copyright © 2001 Nature Publishing Group. Used with permission.]

The dopamine axons form symmetric synapses onto the shafts of the dendritic spines of medium spiny neurons. Here they are well placed to modulate the incoming activity from corticostriatal glutamatergic afferents that form asymmetric synapses onto the heads of the spines (Smith and Bolam, 1990) (see Fig. 4a). There is reciprocal modulation of glutamate and dopamine inputs at the presynaptic level, by transmitter spillover from the synapse acting via dopamine D2 and metabotropic glutamate receptors, respectively (Wang and Pickel, 2002; Zhang and Sulzer, 2003). Indirect glutamatergic influence mediated by diffusible messengers has also been proposed (Avshalumov et al., 2008). It is at this presynaptic level that nicotine exerts its major influence in the striatum.

ACh in the striatum is derived from a population of giant, aspiny cholinergic interneurons, whose diameter can reach 40 μm (Fig. 2). Although they represent less than 2% of the total neuronal population of the striatum, these large cells have an extensive network of processes, enabling them to affect activity throughout the striatum (Wilson and Groves, 1980; Calabresi et al., 2000). It has been estimated that there are only 40,000 cholinergic interneurons in each rat striatum, but each of these neurons

forms half a million varicosities within a territory of up to 1 mm in diameter (Zhou et al., 2002; Tepper and Bolam, 2004). Thus throughout the striatum there is extensive overlap with the dopaminergic arborization that facilitates their cross-talk (Fig. 2). There is evidence for cholinergic synapses on distal dendrites and dendritic spines within the striatum, but ACh is also released nonsynaptically from varicosities to exert effects by volume transmission (Descarries et al., 1997). The cholinergic interneurons are tonically active, firing action potentials at a regular, slow rate (3–10/s) that results in a continuous pulsatile release of ACh under basal conditions (Wilson et al., 1990). The high levels of acetylcholinesterase present in the striatum facilitate the rapid hydrolysis of ACh, enabling extracellular ACh to reflect its pulsatile release, thus minimizing receptor desensitization (Zhou et al., 2001). This contrasts with the concentration profile achieved by exogenous drugs such as nicotine.

The dopamine afferents also exhibit a tonic activity at rest (the regular firing of single action potentials that constitutes a rhythmic pacemaker function). This results in a continuous “drip feed” of dopamine that maintains a tonic concentration of 10 to 20 nM in the striatum (Goto et al., 2007). Thus, spillover of dopamine and ACh released under resting conditions facilitates their interaction. Activa-

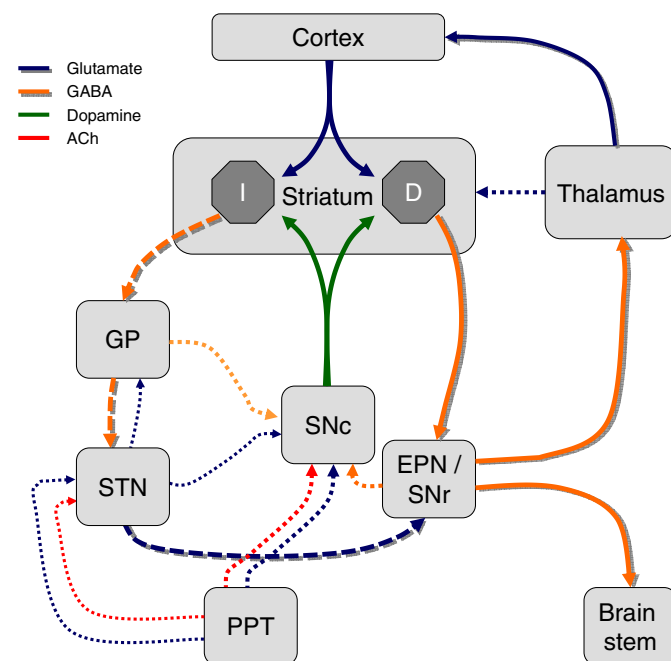


FIG. 3. Schematic of the nigrostriatal pathway in relation to the basic circuitry of the basal ganglia. Dopaminergic neurons of the SN pars compacta and corticostriatal glutamatergic neurons converge on the medium spiny neurons of the striatum. These are the principal output neurons of the “direct” (D) or “indirect” (I) pathways. The direct pathway (heavy shaded lines) projects directly to the entopeduncular nucleus (EPN; internal segment of the globus pallidus in primates) or the SN pars reticulata (SNr), and thence to the thalamus or brain stem, respectively. The indirect pathway (heavy dashed shaded lines) makes synaptic connections in the globus pallidus (GP; external segment of the globus pallidus in primates) and subthalamic nucleus (STN) en route to the EPN and SNr. Some additional connections are shown as dotted lines. See Bolam et al. (2000) for details.

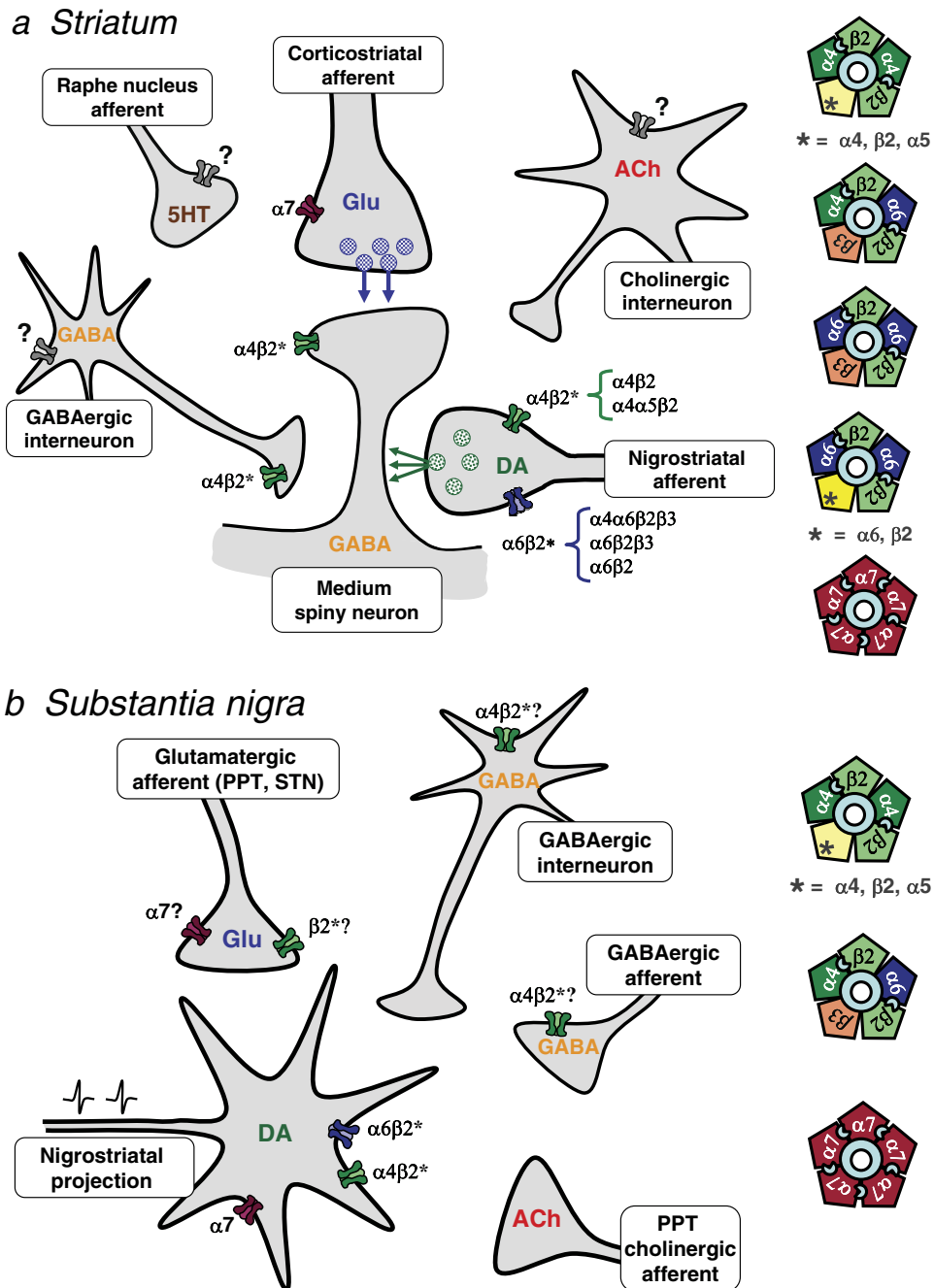


FIG. 4. Cellular localization of nAChR subtypes in striatum (a) and SN (b). a, in the striatum, the nigrostriatal and corticostriatal afferents converge on the shafts and heads, respectively, of the spines of medium spiny projection neurons. The nigrostriatal dopaminergic terminals (DA) bear a variety of $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR subtypes. $\alpha 7$ nAChRs are proposed to reside on the glutamatergic terminals (Glu). Other neuronal elements in the striatum, the GABAergic and cholinergic interneurons and serotonergic afferents from the raphe nucleus, are also indicated. GABAergic terminals express $\alpha 4\beta 2^*$ nAChRs: these may arise from interneurons, as illustrated, or from axon collaterals, medium spiny neurons, or globus pallidus neurons (not shown). A fast-spiking subpopulation of GABAergic interneurons may express an unidentified subtype of nAChR, possibly $\alpha 7$ (gray receptor). Evidence for the presence of nAChRs on cholinergic interneurons and serotonergic afferents is inconclusive (gray receptor). The subunit composition of nAChR subtypes expressed in the striatum is illustrated in the right panel. Two agonist-binding sites are indicated at the interface between α and $\beta 2$ subunits in heteromeric nAChRs, whereas $\alpha 7$ nAChRs have five putative binding sites. b, in the SN, the dopaminergic neurons (DA) that project to the striatum are modulated by glutamatergic, cholinergic, and GABAergic afferents, as well as GABAergic interneurons. The dopamine neurons bear $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR subtypes that may be distinct from those expressed on the striatal terminals. Proposed subunit combinations are illustrated on the right. $\alpha 7$ nAChRs are also present on a proportion of these cell bodies, in contrast to the striatal dopaminergic terminals. GABAergic interneurons express heteromeric nAChRs; $\alpha 4\beta 2^*$ nAChRs may also be present on GABAergic afferents, for example from the substantia nigra pars reticulata. In contrast to the VTA, glutamatergic afferents may bear both $\alpha 7$ and non- $\alpha 7$ nAChRs, but cholinergic afferents are apparently devoid of nAChRs.

tion of midbrain dopamine neurons switches their activity to a “bursting” firing pattern, a phasic pattern of bursts of action potentials (Grace et al., 2007). The consequence is

substantially greater dopamine release in the striatum, transiently achieving high micromolar or even millimolar levels (Goto et al., 2007). Burst firing of dopamine neurons

is accompanied by silencing of the cholinergic interneurons, so ACh release ceases when dopamine release increases. This coordinated reciprocal response, which relies on both nigrostriatal and thalamic inputs to the striatum, emphasizes the complex inter-relationship between dopamine and cholinergic systems in this region (Zhou et al., 2002; Cragg, 2006). The dopamine terminals have a rich array of nAChR subtypes, whereas there is no consistent evidence for nicotinic autoreceptors on the cholinergic interneurons (see section III.A.3). The striatum also receives serotonergic afferents from the raphe nucleus that have elicited recent interest with respect to their role in motor control in the normal brain and in Parkinson's disease (Di Matteo et al., 2008).

Although outside the scope of this review, it is important to recognize that ACh has another target receptor, the muscarinic receptor, that greatly outnumbers nAChRs (Conn et al., 2009). The G-protein-coupled muscarinic receptor exerts a modulatory influence via facilitatory M1-type receptors and inhibitory M2-type receptors, located both presynaptically on corticostriatal and nigrostriatal afferents and somatodendritically on the medium spiny neurons. Inhibitory muscarinic autoreceptors are also present on the cholinergic interneurons to regulate ACh release (Calabresi et al., 2000). In Parkinson's disease, muscarinic antagonists were one of the first treatments and are still sometimes used in a secondary role (Langmead et al., 2008; Conn et al., 2009). Their efficacy is attributed to a reduction in 1) the overactivity of the cholinergic interneurons and 2) the hyperactivity of corticostriatal glutamate neurotransmission that ensues after nigrostriatal denervation. Although they provide some benefit, these drugs are not without side effects, including cognitive impairment. Hence, there is a need for new and improved therapeutics. Any nicotinic agonist therapy will differ from the actions of ACh in its selectivity for nAChRs as well as in its extended pharmacokinetics compared with the highly regulated release of ACh.

B. Substantia Nigra

The dopaminergic neurons projecting to the dorsal striatum are primarily responsible for modulating motor functions and also cognitive aspects of motor learning (Kreitzer and Malenka, 2008). These arise from the A9 group of midbrain dopaminergic neurons, corresponding to the SN pars compacta (Fig. 1). The presence of neuromelanin in these cells gives them their eponymous dark coloration. The number of dopaminergic neurons in each SN pars compacta is estimated at ~ 7000 per side in the mouse, $\sim 12,000$ in the rat, up to 100,000 in monkeys, and more than 200,000 in young humans (Björklund and Dunnett, 2007). In addition to the predominant striatal innervation, some SN neurons innervate cortical and limbic areas. Dopamine is also released locally within the SN and VTA; this somatodendritic release can be modulated by nAChRs (Cheramy et al., 1981; Rahman et al., 2004a). The dendritic arborization extends into the underlying SN pars

reticulata, populated with GABAergic neurons. Thus, dendritically released dopamine can influence the activity of dopamine projection neurons themselves via somatodendritic autoreceptors and GABAergic activity within the SN pars reticulata, which forms the output pathway of the direct and indirect circuits (Fig. 3) (Robertson, 1992; Zhou et al., 2009).

The SN pars compacta contains a population of GABAergic interneurons, as well as GABAergic afferents from the striatum, globus pallidus, and SN pars reticulata, that act as a brake on dopaminergic cell activation. Both the GABAergic and dopamine neurons bear nAChRs (Misgeld, 2004) (see section III.A.2; Fig. 4b). As already noted, midbrain dopamine neurons exhibit two distinct firing patterns, tonic single-spike activity and burst spike firing (Goto et al., 2007). The switch to burst firing is dependent on glutamatergic innervation, which comes from the subthalamic nucleus and the pedunculopontine nucleus (PPT) (Lee and Tepper, 2009). These glutamatergic nerve terminals may also be endowed with nAChRs (Keath et al., 2007).

C. Pedunculopontine Nucleus

The cholinergic input that provides the ACh to interact with nAChRs in the SN pars compacta comes from the PPT situated in the pons (Figs. 1 and 3). Analogous cholinergic innervation of the VTA is from the related laterodorsal tegmental nucleus. The PPT provides both cholinergic and glutamatergic inputs that synapse onto nigral dopamine neurons (Mena-Segovia et al., 2008) (Figs. 3 and 4B). The convergence of these inputs has raised the possibility that both neurotransmitters may be coreleased from the same terminals, with some evidence that this does occur in the squirrel monkey but not in the rat (Lavoie and Parent, 1994; Wang and Morales, 2009). Stimulation of the PPT elicits burst firing in SN pars compacta dopamine neurons (Lokwan et al., 1999; Floresco et al., 2003) and evokes dopamine release in the striatum, which is inhibited by application of either nicotinic or glutamatergic receptor antagonists into the SN pars compacta (Futami et al., 1995; Forster and Blaha, 2003). Indeed, it has been suggested that cholinergic activation is critical for promoting burst firing (Kitai et al., 1999). This idea resonates with a proposal for the VTA (based on studies with knockout mice lacking particular nAChR subunits) that cholinergic nicotinic activation of dopamine cell bodies serves as a gate that facilitates the switch to burst firing, enabling the dopamine neurons to respond to glutamatergic signals (Maskos, 2008, 2010).

III. Neuronal Nicotinic Acetylcholine Receptors

A. Structure and Heterogeneity

nAChRs are pentameric ligand-gated cation channels, permeable to Na^+ , K^+ , and, to varying degrees, Ca^{2+} . The five membrane-spanning subunits create a central pore or channel that is opened in response to binding ACh or

exogenous agonist. Muscle-type nAChRs, found in skeletal muscle and in *Torpedo* spp. electric tissues, have provided detailed insights into the structure and function of the receptor (Unwin, 2003). However, a distinct set of genes coding for neuronal nAChR subunits is expressed in neurons and some non-neuronal cells, including glia (Albuquerque et al., 2009; Millar and Gotti, 2009). To date, nine neuronal nAChR subunit genes have been shown to be expressed in various mammalian CNS neurons ($\alpha 2$ – $\alpha 7$; $\beta 2$ – $\beta 4$). The most abundant and widespread of these are $\beta 2$, $\alpha 4$, and $\alpha 7$. Others have a more restricted distribution; for example, expression of $\alpha 6$ and $\beta 3$ subunits is largely limited to catecholaminergic neurons. In addition, $\alpha 8$ has been found only in avian systems, and $\alpha 9$ and $\alpha 10$ are limited to cochlear hair cells, sensory neurons, and some non-neuronal cells. In theory, these subunits could combine to give a huge array of nAChR subtypes. However, the diversity of native nAChR subtypes is more limited, and assembly into viable nAChRs seems to be constrained by a set of presently poorly understood rules.

The simplest subunit combination is a pentamer of identical subunits (Albuquerque et al., 2009; Millar and Gotti, 2009). Of the subunits expressed in the mammalian brain, only the $\alpha 7$ subunit is able to form homomeric nAChRs in expression systems. Most, if not all, native $\alpha 7$ nAChRs are also homomers (Séguéla et al., 1993). In contrast, $\alpha 2$ – $\alpha 6$ subunits are incapable of forming homomeric receptors and require β subunits (and additional α subunits in the case of $\alpha 5$) for formation of functional nAChRs (Albuquerque et al., 2009; Millar and Gotti, 2009). The agonist binding site occurs primarily on α subunits (“principal binding site”) but is formed at the interface with the adjacent β subunit that also contributes complementary binding residues (“complementary binding site”) (Corringer et al., 2000; Celie et al., 2004). For heteromeric neuronal nAChRs, $\alpha 2$, $\alpha 3$, $\alpha 4$, and $\alpha 6$ subunits can pair with $\beta 2$ or $\beta 4$ subunits to create an agonist binding site (see Fig. 4). As demonstrated for muscle-type nAChR, it is assumed that two binding sites per nAChR must be occupied for effective opening of the ion channel. In contrast to $\beta 2$ and $\beta 4$, the $\beta 3$ subunit does not contribute to binding sites but is regarded as an “accessory subunit” that occupies the fifth position in the nAChR, analogous to the $\beta 1$ subunit in muscle nAChR. The $\alpha 5$ subunit also functions exclusively as an accessory subunit, in that it lacks key residues critical for agonist binding (Kuryatov et al., 2008). Other α and β subunits can occupy the fifth position and form $\alpha\beta$ agonist binding pairs (Fig. 4). Subunit composition determines nAChR properties, including channel open time, ion permeability and selectivity, and rate of desensitization, in addition to agonist sensitivity (Albuquerque et al., 2009; Millar and Gotti, 2009).

The structural diversity of nAChRs is paralleled by the diversity of their localization (at both cellular and tissue levels). Although this review is focused on nAChRs within the basal ganglia, it is important to emphasize that nAChR subtypes occur throughout the central and peripheral ner-

vous systems, as well as on some non-neuronal cells. Thus nAChRs influence many physiological mechanisms, including pain, inflammation, cognition, and others (Bacher et al., 2009; Buckingham et al., 2009; McIntosh et al., 2009; Poorthuis et al., 2009; Sarter et al., 2009; Changeux, 2010a; Mineur and Picciotto, 2010; Philip et al., 2010). As a consequence, systemic nicotine and other agonists have many and varied biological effects, beyond the modulation of motor control.

1. Pharmacological Tools to Study Nicotinic Acetylcholine Receptor Subtypes in the Nigrostriatal System

A host of experimental approaches has been used to identify and study the different nAChR subtypes described in the previous section, including mRNA work, immunoprecipitation with selective nAChR subunit-directed antibodies, and the use of genetically engineered mice. In addition, pharmacological tools have assisted in identification and in characterization of distribution and function. Some of the more common drugs used as investigational tools are described below.

A variety of different agonists and antagonists have been used to study CNS $\alpha 4\beta 2^*$ nAChRs (* signifies the possible presence of other subunits in the nAChR complex). However, emerging studies suggest that many of these compounds also act at other nAChR subtypes, particularly $\alpha 6\beta 2^*$ nAChRs. Thus, mecamylamine and dihydro- β -erythroidine, two antagonists frequently used to investigate $\alpha 4\beta 2^*$ nAChR-mediated function, also block $\alpha 6\beta 2^*$ nAChRs (Exley et al., 2008; Meyer et al., 2008; Perez et al., 2008). With respect to $\alpha 4\beta 2^*$ nAChR-directed agonists, 3-[(2*S*)-2-azetidylmethoxy]-5-iodopyridine dihydrochloride (5-iodo-A-85380) was initially reported to selectively bind to $\alpha 4\beta 2^*$, with much lower affinity for $\alpha 3\beta 4^*$ and $\alpha 7$ nAChRs (Mukhin et al., 2000). However, it was subsequently shown to act with similar potency at both $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs (Kulak et al., 2002b). Varenicline, a partial $\alpha 4\beta 2^*$ nAChR agonist, also interacts with $\alpha 3\beta 4^*$, $\alpha 6\beta 2^*$, and $\alpha 7$ nAChRs (Coe et al., 2005; Gonzales et al., 2006; Jorenby et al., 2006; Rollema et al., 2007a,b; M. Quik, unpublished observations). Sazetidine-A, another agent initially reported as selective for $\alpha 4\beta 2^*$ receptors, binds with high affinity to $\alpha 6\beta 2^*$ nAChRs and stimulates both $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR-mediated dopamine release (Xiao et al., 2006; Cucchiari et al., 2008; Zwart et al., 2008; M. Quik, unpublished observations). In addition, the agonist ABT-089 (pozanicline) (Sullivan et al., 1997) has activity at both $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ receptors (Marks et al., 2009). Numerous other drugs in the literature have also been reported to interact with $\alpha 4\beta 2^*$ nAChRs, including undesigned compounds from Abbott Laboratories (Abbott Park, IL), Targacept, Inc. (Winston-Salem, NC), SIBIA Neurosciences (La Jolla, CA), and University of Bath (Bath, UK). However, at this point, their selectivity is uncertain because their interaction with $\alpha 6\beta 2^*$ nAChRs and/or other nAChR subtypes is not known (Bencherif et al., 1996; Cosford et al., 1996; Donnelly-Roberts et al., 1996, 1998; Bencherif et al., 2000; Sharples

et al., 2000; Buccafusco et al., 2005; Gotti et al., 2006b; Lippiello et al., 2006; Wang et al., 2006; Dwoskin and Bardo, 2009). All together, these observations indicate that the majority of available $\alpha 4\beta 2^*$ nAChR drugs may interact with other nAChR subtypes, notably $\alpha 6\beta 2^*$ nAChRs; at best these agents should be regarded as only $\beta 2$ -selective. However, general $\beta 2$ -selective agonists may have therapeutic advantages, as discussed in section VIII.B (Huang et al., 2011).

A toxin that has proved invaluable in elucidating the nature and function of $\alpha 6\beta 2^*$ nAChRs is α -conotoxinMII (McIntosh et al., 2004; Quik and McIntosh, 2006). This 16-amino acid peptide, originally isolated from the venom of the marine snail *Conus magus*, selectively interacts at $\alpha 3\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs (Cartier et al., 1996; Champ-tiaux et al., 2002). Because there is little evidence for the existence of $\alpha 3\beta 2^*$ nAChRs in mouse brain (Whiteaker et al., 2002), α -conotoxinMII provides information specifically concerning $\alpha 6\beta 2^*$ nAChR in the rodent nigrostriatal pathway. By contrast, $\alpha 6\beta 2^*$ and a small population of $\alpha 3\beta 2^*$ nAChRs are present in monkey striatum; thus, α -conotoxinMII would interact at both subtypes (Quik et al., 2005). Another toxin, α -conotoxinPIA from *Conus purpurascens*, can discriminate between $\alpha 6^*$ and $\alpha 3^*$ nAChRs (Dowell et al., 2003; Gotti et al., 2010) but it is not readily accessible. No other toxins, drugs, or agents presently exist that selectively interact with $\alpha 3\beta 2^*$ and/or $\alpha 6\beta 2^*$ nAChRs. Thus, discrimination of native nAChRs that have minor differences in subunit composition is currently not possible.

Because selective agonists for $\alpha 7$ nAChRs were not available until more recently, antagonists have been particularly important for the study of these receptors. A key antagonist used to study $\alpha 7$ nAChRs is α -bungarotoxin. This toxin, isolated from the venom of *Bungarus multicinctus*, binds to $\alpha 1^*$, $\alpha 7$, and $\alpha 9/10$ nAChRs (Albuquerque et al., 2009). Because $\alpha 1^*$ receptors are present only in skeletal muscle, and $\alpha 9/10$ receptors are not found in the brain, α -bungarotoxin has proved to be a very selective tool for $\alpha 7$ nAChRs in the CNS. It is used primarily for in vitro studies because it does not cross the blood-brain barrier, although it can be injected intracerebrally. Methyllycaconitine is a small-molecular-weight $\alpha 7$ nAChR antagonist that readily enters the brain when given systemically (Macallan et al., 1988; Ward et al., 1990). However, although selective for $\alpha 7$ nAChRs at low nanomolar concentrations, it interacts with other nAChR subtypes, notably $\alpha 6\beta 2^*$ nAChRs, at higher concentrations (Mogg et al., 2002). Small-molecular-weight $\alpha 7$ nAChR agonists include 3-[(3*E*)-3-[(2,4-dimethoxyphenyl)methylidene]-5,6-dihydro-4*H*-pyridin-2-yl]pyridine (GTS-21), (4-bromophenyl)-1,4-diazabicyclo [3.2.2]nonane-4-carboxylate (SSR180711), *N*-(3*R*)-1-azabicyclo[2.2.2]oct-3-yl-4-chlorobenzamide (PNU-282987), and (2*S*)-2'*H*-spiro[4-azabicyclo[2.2.2]octane-2,5'-[1,3]oxazolidin]-2'-one (AR-R17779) (Levin et al., 1999; Simosky et al., 2002; Martin et al., 2004; Hansen et al., 2007; Söderman et al., 2011). Choline, the breakdown product of ACh, is also found to selectively activate $\alpha 7$ nAChRs at millimolar concentrations (Alkondon et

al., 1997). Ambient levels of choline, especially in areas such as striatum, where there are tonically active cholinergic neurons, might be sufficient to maintain $\alpha 7$ nAChRs in a desensitized state. The recent generation of allosteric potentiators, some specific for $\alpha 7$ nAChRs, are valuable additions to the toolbox for enhancing or revealing the contribution of $\alpha 7$ nAChRs (Bertrand and Gopalakrishnan, 2007).

2. Nicotinic Acetylcholine Receptor Subtypes in the Substantia Nigra

Determining the cellular expression of nAChR subunits and the subcellular localization of defined nAChR subtypes poses a major challenge. However, this has proved somewhat easier to address for the nigrostriatal dopamine pathway than for other brain systems, because dopamine afferents are highly localized to this ascending pathway, which can be selectively lesioned by dopaminergic neurotoxins. As well, the striatum and SN are relatively homogeneous with respect to their neurochemical makeup and neuronal composition.

In rodents, mRNA for five nAChR subunits, ($\alpha 4$, $\alpha 5$, $\alpha 6$, $\beta 2$, $\beta 3$) is expressed at high levels in the SN pars compacta, with lower levels of $\alpha 7$ mRNA (Wada et al., 1989; Marks et al., 1992; Cui et al., 2003). The dopamine neurons and GABAergic interneurons that populate the SN pars compacta can be distinguished by expression of tyrosine hydroxylase or glutamate decarboxylase, respectively, and by their characteristic electrophysiological properties, such as intrinsic membrane potential and firing properties (Grace and Bunney, 1983a,b,c; Lacey et al., 1989). Single-cell reverse transcription-polymerase chain reaction or double-labeling in situ hybridization in combination with these indices of neuronal identity showed that most dopamine neurons express mRNA corresponding to $\alpha 4$, $\alpha 5$, $\alpha 6$, $\beta 2$, and $\beta 3$. At a lower level and in a lower proportion of cells, $\alpha 3$ nAChR mRNA was also detected (Klink et al., 2001; Azam et al., 2002). However, $\alpha 3$ subunit expression decreased during development in the rat; this decrease coincided with an increase in $\alpha 6$ subunit expression (Azam et al., 2007). In contrast, SN GABA neurons displayed a simpler expression pattern largely restricted to $\alpha 4$, $\beta 2$, and $\alpha 3$. In addition, approximately 40% of both dopamine and GABA neurons of the SN expressed mRNA for $\alpha 7$ nAChRs (Klink et al., 2001). $\beta 4$ mRNA expression was generally low, but higher in nondopaminergic neurons (Klink et al., 2001; Azam et al., 2002). Overall, the pattern of nAChR transcript expression in nonhuman primates resembles that in rodents, except for the $\alpha 2$ mRNA, which is absent in the rodent but present in the primate SN (Han et al., 2000; Quik et al., 2000a,b).

The next challenges were to determine 1) the subunit combinations forming native, functional nAChRs in these neurons and 2) their subcellular disposition (Fig. 4b). Klink et al. (2001) used pharmacological tools and transgenic mice lacking $\alpha 4$ or $\alpha 7$ subunits to analyze nicotinic currents elicited from cell bodies of SN dopamine neurons. They interpreted their data in favor of two predominant

somatodendritic subtypes: $\alpha 4\alpha 6(\beta 2)_2\alpha 5$ (sensitive to blockade by α -conotoxinMII) and $(\alpha 4)_2(\beta 2)_2\alpha 5$ (insensitive to α -conotoxinMII). The former subtype is distinct from any of those identified in striatum (see below), although more recent immunoprecipitation studies found no evidence for the association of $\alpha 5$ with $\alpha 6$ subunits in midbrain; thus, $\alpha 4\alpha 6(\beta 2)_2\beta 3$ may be a more likely subunit composition (Gotti et al., 2010). However, Gotti et al. (2010) noted the different complement of nAChR subunit combinations identified in midbrain compared with striatum, suggesting the occurrence of some different nAChR subtypes in somatodendritic and terminal compartments. In addition, typical $\alpha 7$ nAChR-mediated currents could be elicited by choline in the lower proportion of dopaminergic neurons (Klink et al., 2001; Keath et al., 2007).

There is little functional evidence that addresses the subunit composition of nAChR on the sparse GABA neurons in the SN pars compacta, although both $\alpha 7$ and non- $\alpha 7$ nAChRs have been localized to the soma and proximal dendrites of GABAergic neurons of the SN pars reticulata (Poisik et al., 2008). In the VTA, the absence of functional responses (other than $\alpha 7$ nAChR responses) in GABAergic neurons in $\alpha 4$ or $\beta 2$ knockout mice led to the proposition that non- $\alpha 7$ nAChRs are $\alpha 4\beta 2$ and possibly $\alpha 3\alpha 4\beta 2$ subtypes (Klink et al., 2001). However, in SN GABA neurons, nAChR subunit expression is more diverse (Klink et al., 2001), and nAChRs on GABA neurons exhibit a different pharmacological profile compared with VTA (Keath et al., 2007), leaving some uncertainty about the exact subunit composition and stoichiometry of the nAChRs present. The conclusion that GABA interneurons in the SN pars compacta, as well as the projection neurons of the SN pars reticulata, are endowed with heteromeric nAChRs that have distinct properties compared with those expressed on dopamine cell bodies is consistent with functional studies described in section III.B.1.

In summary, a large portfolio of nAChR subunits is expressed in the SN pars compacta, which contains the dopaminergic cell bodies. Multiple complex subtypes of nAChR seem to exist in these dopamine neurons, whereas GABA interneurons exhibit a somewhat simpler expression pattern (Fig. 4b). The presence of distinct nAChR subtypes on dopaminergic and GABAergic neurons in the SN has functional implications, in that it may allow nAChR drugs with differing pharmacodynamic profiles to interact preferentially with one or other of these cell types.

3. Nicotinic Acetylcholine Receptor Subtypes in the Striatum

Identification of nAChR subtypes present on dopamine axon terminals projecting to the striatum could not be undertaken at the single cell level. Instead, neurochemical assays of nAChR function have commonly been employed (Grady et al., 2007). These studies have also relied on subtype-selective pharmacological tools and knockout mice, complemented by immunoprecipitation assays using subtype-specific antibodies to pull down nAChR complexes labeled with a radioligand, typically [3 H]epibatidine (Gotti

et al., 2007). Selective lesioning of the nigrostriatal pathway using 6-hydroxydopamine or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to eliminate dopamine terminals has been used to distinguish this population (Zoli et al., 2002; Quik et al., 2007b). As a whole, this substantial body of work has generated a comprehensive and coherent picture of nAChR subtypes expressed on dopamine terminals, and the heterogeneity is surprising.

First, $\alpha 3$ and $\alpha 7$ subunits do not contribute to presynaptic nAChRs on dopamine terminals in the rodent striatum, despite their expression by some SN pars compacta neurons and their contribution to the population of nAChRs on SN cell bodies (Whiteaker et al., 2002). Conversely, $\beta 3$ has been credited with contributing only to presynaptic $\alpha 6^*$ nAChRs (Zoli et al., 2002; Cui et al., 2003; Salminen et al., 2004) and may be important for targeting or trafficking nAChRs that include it (Tumkosit et al., 2006). Immunoprecipitation studies found modest amounts of $\alpha 4\alpha 6\beta 2\beta 3$ nAChRs in SN, as discussed above (Gotti et al., 2010); it is not known whether these represent functional somatodendritic receptors or nAChRs destined to be trafficked to the striatum. Absence of the $\beta 3$ subunit in null mutant mice reduces but does not eliminate $\alpha 6^*$ nAChRs (Gotti et al., 2005). Although this observation suggests that $\beta 3$ is not an obligatory partner of $\alpha 6$, the two subunits have been seen to decrease in parallel after lesions of the nigrostriatal pathway, which would suggest that they are tightly correlated (Zoli et al., 2002; Quik et al., 2003). A parsimonious explanation is that $\beta 3$ normally combines with $\alpha 6$ for the most efficient assembly, stabilization, and/or trafficking of $\alpha 6\beta 3^*$ nAChRs, and in its absence only low levels of inefficiently processed $\alpha 6^*$ nAChRs are maintained. The asterisk signifies the possible presence of other subunits in the nAChR complex (see Fig. 4a).

The subunits that are present in presynaptic nAChRs on dopaminergic terminals in rodent striatum include $\alpha 4$, $\alpha 5$, $\alpha 6$, $\beta 2$, and $\beta 3$. These comprise 5 subtypes that have been subdivided into two main categories based on their interaction with the snail toxin α -conotoxinMII (McIntosh et al., 1999; Whiteaker et al., 2000) (Fig. 4a). These include those containing the $\alpha 6$ subunit ($\alpha 4\alpha 6\beta 2\beta 3$, $\alpha 6\beta 2\beta 3$, $\alpha 6\beta 2$), which are termed α -conotoxinMII-sensitive because they bind α -conotoxinMII. The other class ($\alpha 4\beta 2$, $\alpha 4\alpha 5\beta 2$ subtypes) does not contain $\alpha 6$, does not bind α -conotoxinMII, and is designated α -conotoxinMII-insensitive (Zoli et al., 2002; Salminen et al., 2004). Functional measurements have suggested a greater proportion of $\alpha 6\beta 2^*$ nAChRs in the nucleus accumbens compared with striatum (caudate putamen) (Exley and Cragg, 2008).

nAChR subtype heterogeneity could be further increased by $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs existing in two stoichiometric forms, as has been shown for heterologously expressed $\alpha 4\beta 2^*$ nAChRs (Nelson et al., 2003; Moroni et al., 2006) (Fig. 4a). These alternate forms have distinct agonist sensitivities depending on whether $\alpha 4$ or $\beta 2$ occupies the fifth, accessory position; that is, $(\alpha 4)_2(\beta 2)_3$ displays

higher affinity for ACh and nicotine than $(\alpha 4)_3(\beta 2)_2$. With regard to the regulation of dopamine release from striatal terminals, pharmacological evidence in favor of the high-sensitivity $(\alpha 4)_2(\beta 2)_3$ form (Anderson et al., 2009) or both high- and low-sensitivity forms (Grady et al., 2010a) has been presented. The interpretation of data for native subtypes is complicated by the high affinity shown by $(\alpha 4)_2(\beta 2)_2\alpha 5$ nAChRs that are also present on dopamine terminals (Grady et al., 2010a). The occurrence and functional significance of alternative stoichiometries of $\alpha 6\beta 2$ nAChRs has not yet been elucidated.

The proportion of α -conotoxinMII-sensitive to insensitive nAChR subtypes is $\sim 30:70$ in rodents (Cartier et al., 1996; Kulak et al., 1997; Grady et al., 2007) but this ratio is $\sim 50:50$ in monkey striatum (Kulak et al., 2002). This might reflect the additional presence of α -conotoxinMII-sensitive $\alpha 3\beta 2^*$ nAChR on nigrostriatal terminals in monkey brain (Quik et al., 2005). After nigrostriatal lesions with either MPTP or 6-hydroxydopamine, $\alpha 6$ and $\beta 3$ subunit-containing nAChRs decline in parallel with the loss of dopaminergic markers such as the dopamine transporter (Zoli et al., 2002; Champtiaux et al., 2003; Cui et al., 2003; Quik et al., 2003, 2005). By contrast, nAChRs composed of $\alpha 4$ and $\beta 2$ (but not $\alpha 6$) subunits, which can be detected by their ability to bind agonist with high affinity in the presence of α -conotoxinMII, are much less affected by lesioning in both rodents and primates, such that up to 70% of $\alpha 4\beta 2^*$ nAChRs may be spared (Quik et al., 2001; Kulak et al., 2002a; Zoli et al., 2002; Champtiaux et al., 2003; Quik et al., 2003). An explanation of this anomaly is that expression of the $\alpha 6$ and $\beta 3$ subunits is restricted to catecholaminergic neurons, whereas $\alpha 4\beta 2^*$ nAChRs also occur on nondopaminergic components of the striatum. However, the high level of residual $\alpha 4\beta 2^*$ nAChRs is surprising. The paucity of *in situ* hybridization signals for expression of nAChR subunits in striatum or caudate putamen is striking (Wada et al., 1989; Marks et al., 1992; Le Novère et al., 1996) and reinforces the notion that the majority of nAChRs in this region is located presynaptically on afferents rather than expressed by intrinsic cells. There is neurochemical evidence for $\alpha 7$ nAChRs on glutamate afferents (Kaiser and Wonnacott, 2000; Marchi et al., 2002). nAChRs accounting for 30% of the striatal population of [^3H]ACh binding sites (that equate with $\alpha 4\beta 2^*$ nAChRs) were reported to occur on serotonin terminals (Schwartz et al., 1984), but this could not be reproduced in a subsequent study (Pradhan et al., 2002). However, nAChRs with a novel pharmacological profile were ascribed to serotonin terminals based on transmitter release studies from synaptosomes (Reuben and Clarke, 2000). The relationship between nAChRs and serotonergic terminals warrants clarification in view of recent evidence for the sprouting of these afferents in L-DOPA-induced dyskinesia (Rylander et al., 2010).

Medium spiny projection neurons seem to be devoid of nAChRs (Jones et al., 2001; Zhou et al., 2002) but fast-spiking GABA interneurons (which comprise a tiny pro-

portion of the total neuronal population) were shown to respond to the application of ACh or carbachol in a manner consistent with somatodendritic (possibly extrasynaptic) nAChRs (Koós and Tepper, 2002). In an elegant study, photoactivation of optogenetically engineered cholinergic interneurons of the nucleus accumbens resulted in an increased frequency of inhibitory currents in medium spiny neurons, and this response was sensitive to mecamylamine (Witten et al., 2010). The network connections mediating the increased firing rate are unclear, but it is plausible that GABAergic interneurons bearing nAChRs are involved, rather than a direct cholinergic action on medium spiny neurons.

There is also evidence for functional $\alpha 4\beta 2^*$ (including $\alpha 4\alpha 5\beta 2$) nAChRs on GABAergic terminals from striatum (Grilli et al., 2009; McClure-Begley et al., 2009). These could arise from GABA interneurons, axon collaterals from the medium spiny projection neurons or collateral projections from the GABAergic neurons in the globus pallidus (Bolam et al., 2000).

Cholinergic interneurons might express $\beta 2$ subunit mRNAs, because $\beta 2$ -like immunoreactivity was reported in "sparsely distributed large neurons" in the rat striatum that might correspond to cholinergic interneurons (Hill et al., 1993; Azam et al., 2003). On the other hand, Jones et al. (2001) did not detect any $\beta 2$ immunolabeling of cell bodies in striatum. Functional studies support the presence of muscarinic but not nAChRs on the cell bodies of cholinergic interneurons (Calabresi et al., 1998; Windels and Kiyatkin, 2003). Moreover, there are conflicting reports of presynaptic nicotinic modulation of ACh release in striatum with positive results reported by some (Sandor et al., 1991; Yu and Wecker, 1994) but not others (Araujo et al., 1988).

One study reported MLA-sensitive nicotine-evoked currents, consistent with $\alpha 7$ nAChRs, in a proportion of medium spiny neurons, fast-spiking interneurons, and cholinergic interneurons in mouse striatum (Xiao et al., 2009). This is consistent with the finding of $\alpha 7$ subunit mRNA in some rat striatal cholinergic interneurons (Azam et al., 2003). However, levels of ^{125}I - α -bungarotoxin binding in striatum are generally low, although higher in mouse than rat, indicative of only low levels of this nAChR subtype (Marks et al., 1986).

In summary, dopamine terminals in the striatum express a remarkable diversity of nAChRs, with the $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR subtypes studied most extensively. The $\alpha 6\beta 2^*$ nAChRs are unique to dopamine terminals in striatum, whereas $\alpha 4\beta 2^*$ nAChRs are more promiscuous and are localized to dopamine terminals and other neuronal elements. Much less is known about the latter category of $\alpha 4\beta 2^*$ nAChRs with respect to their subunit composition and cellular localization, but their resistance to nigrostriatal degeneration makes it important to understand this population better. There is also little information about striatal $\alpha 7$ nAChRs. In addition, the role of $\alpha 3\beta 2^*$ nAChRs,

present on nigrostriatal terminals in primates but not found in rodent striatum, remains to be identified.

B. Nicotinic Acetylcholine Receptor Modulation of Dopaminergic Function

1. Substantia Nigra

As mentioned in section II.B, SN dopamine neurons exhibit a tonic pacemaker activity, with regular firing of action potentials (Grace and Bunney, 1984a,b). Cholinergic inputs from the PPT regulate the activity of dopamine neurons (Mena-Segovia et al., 2008). Early studies in rats recognized that systemic nicotine increased the firing rate of SN pars compacta neurons (Lichtensteiger et al., 1982; Clarke et al., 1985) and promoted burst firing (Greenhoff et al., 1986). These findings have been recapitulated in a recent study (Zhang et al., 2009b). Iontophoretic application of ACh in the SN pars compacta also enhanced firing, and this was inhibited by dihydro- β -erythroidine but not atropine, implicating a nicotinic, rather than muscarinic, receptor-mediated response (Lichtensteiger et al., 1982). These studies suggested a direct excitatory action of nicotine on SN dopamine neurons, mimicking the endogenous cholinergic innervation from the PPT (Clarke et al., 1987). However, the excitability of dopamine neurons is restrained by GABAergic inputs and influenced by glutamatergic afferents (Misgeld, 2004; Lee and Tepper, 2009). The presence of nAChRs on these elements as well as on dopamine cell bodies and dendrites (Fig. 4b) presents a more complex scenario for the nicotinic regulation of dopamine cell activity.

The nicotinic regulation of SN pars compacta dopamine cell firing has been studied much less extensively than that in the VTA, reflecting interest in the mesolimbic "reward" pathway with respect to nicotine dependence. This observation also marks another limitation in the field, in that there has been greater emphasis on the actions of the exogenous drug nicotine than on the endogenous cholinergic mechanisms governing physiological function. Nevertheless, given the comparable firing patterns (Zhang et al., 2009b) and similarities in the neurochemical interactions and disposition of nAChRs in the VTA and SN (Livingstone and Wonnacott, 2009) and comparable, albeit pharmacologically distinct, nicotinic modulation in the VTA and SN (Keath et al., 2007), it can be argued that similar mechanisms are likely to operate in the two regions. With these caveats, we can propose a model for the SN pars compacta analogous to that proposed for the VTA (McKay et al., 2007), in which activation of $\beta 2^*$ nAChRs on dopamine neurons increases firing rates. Concomitant desensitization of distinct $\beta 2^*$ nAChRs on GABA interneurons, with a resultant decrease in inhibitory input onto the dopamine cells, would enhance the activation of dopamine neurons.

The propensity of $\beta 2^*$ nAChRs on GABA interneurons to desensitize more rapidly than those on dopamine cell bodies (Yin and French, 2000) is presumed to reflect subtle differences in their subunit composition, local environ-

ment, or cell-specific regulatory mechanisms (Dani et al., 2000). As discussed in section III.A.1, nAChRs on dopamine neurons are more complex in subunit composition and diversity (Fig. 4b). In particular, expression of the $\alpha 6$ nAChR subunit is limited to catecholaminergic neurons, and $\alpha 6\beta 2^*$ nAChRs occur in dopamine but not GABA neurons of the SN (Klink et al., 2001). Indeed, mice with a gain-of-function mutation in the channel-forming M2 segment (L9'S) of the $\alpha 6$ nAChR subunit are hyperactive (Drenan et al., 2008). This behavior is attributed to midbrain dopamine neurons' being rendered hypersensitive to cholinergic activation by endogenous ACh or exogenous nicotine. As this phenotype is also dependent on the presence of $\alpha 4$ nAChR subunits, $\alpha 6\alpha 4\beta 2^*$ nAChRs may be the dominant $\alpha 6\beta 2^*$ receptor subtype in dopamine neurons (Drenan et al., 2010). Thus $\alpha 6\beta 2^*$ nAChRs could account, at least in part, for the differential responses of GABA and dopamine neurons.

Midbrain $\alpha 7$ nAChRs are present on a proportion of dopamine neurons and are also proposed to reside on glutamate terminals (Fig. 4b). In SN pars compacta, activation of these presynaptic $\alpha 7$ nAChR receptors, in concert with non- $\alpha 7$ nAChRs, also presumed to be on glutamate afferents, increases the frequency of spontaneous EPSCs recorded from dopamine neurons (Keath et al., 2007). Only $\alpha 7$ nAChRs have been implicated on glutamate afferents to the VTA (Mansvelder and McGehee, 2000; Placzek et al., 2009), and this distinction may reflect differences in the glutamatergic innervation of the two regions (Misgeld, 2004). Coincident activation of presynaptic $\alpha 7$ nAChRs and postsynaptic depolarization as a consequence of activating somatodendritic $\beta 2^*$ nAChR can induce long-term potentiation in VTA dopamine neurons *in vitro* (Mansvelder and McGehee, 2000). Although $\alpha 7$ nAChRs commonly display fast desensitization, this depends on agonist concentration, and low levels of agonist can elicit more sustained $\alpha 7$ nAChR activity (Papke and Porter Papke, 2002). Moreover, $\alpha 7$ nAChRs exhibit very high relative permeability to Ca^{2+} , and brief Ca^{2+} transients arising from opening of the $\alpha 7$ nAChR can be augmented (spatially and temporally as well as in magnitude), by promoting Ca^{2+} -induced Ca^{2+} release from internal stores (Tsuneki et al., 2000; Dajas-Bailador and Wonnacott, 2004; Fucile, 2004). Such features equip $\alpha 7$ nAChR for a role in synaptic plasticity (Mansvelder and McGehee, 2000; McKay et al., 2007). Glutamate release, and its enhancement via $\alpha 7$ nAChRs, has been implicated in the switch to burst firing in the VTA, mediated by NMDA receptors (Chergui et al., 1993; Overton and Clark, 1997; Schilström et al., 2003). Indeed, the absence of burst firing in slice preparations signifies the importance of afferent inputs for this phenomenon (Grace and Onn, 1989). The SN pars compacta of rodents has relatively fewer $\alpha 7$ nAChRs than the VTA and smaller choline-evoked currents (Wooltorton et al., 2003; Keath et al., 2007), raising some questions over the role of $\alpha 7$ nAChRs in the SN. However, the glutamate afferents to the SN pars compacta, arising principally from the subtha-

lamic nucleus, as well as the PPT, do induce burst firing, mainly via NMDA receptors, in a fashion analogous to that of the prefrontal cortex inputs to the VTA (Lee and Tepper, 2009).

The relative contributions of $\alpha 7$ and $\beta 2^*$ nAChRs in the VTA were explored in an elegant study by Mameli-Engvall et al. (2006). In this study, extracellular single unit recordings were made from the VTA of anesthetized wild-type and null mutant mice lacking the $\beta 2$ or $\alpha 7$ subunit, and interpretations were confirmed by lentiviral re-expression of the absent $\beta 2$ subunit. Spontaneous firing rate and firing pattern in wild-type animals could be classified into four categories: low firing/low bursting, low firing/high bursting, high firing/low bursting, or high firing/high bursting. $\beta 2$ subunits (and hence $\beta 2^*$ nAChRs) were essential for high frequency rates of firing and/or bursting. It is suggested that $\beta 2^*$ nAChRs could provide sufficient depolarization for activation of NMDA receptors, functioning as a "switch" or "gate" between basal and excited states (Mameli-Engvall et al., 2006; Ungless and Cragg, 2006). $\alpha 7$ nAChRs seemed to have a more subtle role, as both low-firing/low-bursting and high-firing/high-bursting states were observed in $\alpha 7$ knockout mice, but the intermediate states were absent (Mameli-Engvall et al., 2006). How presynaptic $\alpha 7$ nAChRs on glutamate afferents versus somatodendritic $\alpha 7$ nAChRs on dopamine neurons differentially shape these responses is unknown.

In summary, the activity of dopamine neurons in the SN pars compacta is driven, in part, by cholinergic input from the PPT. Evidence from the VTA suggests that somatodendritic $\beta 2^*$ nAChRs (notably $\alpha 6\beta 2^*$ nAChRs that are confined to the dopaminergic neurons) mediate the principal effects of ACh, and play a critical, permissive role with respect to facilitating responses to glutamatergic inputs (Maskos, 2008). $\alpha 7$ nAChRs exert a more subtle influence. Presynaptic $\alpha 7$ nAChRs on glutamate afferents may contribute to burst firing and synaptic plasticity. The significance of somatodendritic $\alpha 7$ nAChRs on a proportion of dopamine neurons is presently unclear. The activity of dopamine neurons is constrained by GABA afferents and local interneurons; nicotine (but not tonically released ACh) preferentially desensitizes $\alpha 4\beta 2^*$ nAChRs on GABA interneurons, relieving this inhibition. In addition, bursting in SN pars compacta neurons may also be influenced by the autoinhibitory actions of dendritically released dopamine (Pucak and Grace, 1994), which is also subject to nicotinic modulation (Reuben and Clarke, 2000; Rahman et al., 2004a; Rahman et al., 2004b).

2. Striatum

The consequence of SN pars compacta dopamine neuron activity is the release of dopamine in the striatum. The tonic firing of single action potentials (typically at a frequency of 1–5 Hz) maintains low, nanomolar concentrations of extracellular dopamine (Goto et al., 2007). Burst firing, which accompanies behaviorally salient stimuli, produces proportionately much greater release of dopamine, achieving local transient concentrations in the mi-

cro-molar to millimolar range. As discussed in section II.C, cholinergic input from the PPT is a determinant of burst firing, and direct stimulation of the PPT elicits striatal dopamine release via nigral nicotinic and glutamate receptors (Forster and Blaha, 2003). Thus, physiologically, striatal dopamine release is largely driven by action potentials generated in the cell bodies of the SN pars compacta. However, levels of extracellular dopamine also depend on terminal release efficiency, diffusion and spillover from the synapse versus reuptake by dopamine transporters, and regulation via autoreceptors and other inputs (Zhang et al., 2009a). It is in this context that striatal nAChRs contribute.

Dopaminergic terminals in the striatum are well endowed with a distinct population of $\beta 2^*$ nAChRs (Fig. 4a), and much attention has focused on nAChR-mediated dopamine release in vitro. The use of striatal synaptosome or chopped tissue preparations clearly demonstrated the ability of presynaptic nAChRs to promote Ca^{2+} -dependent dopamine release in the absence of any other depolarizing stimulus (Wonnacott, 1997; Grady et al., 2007). Using knockout mice lacking specific subunits and pharmacological tools, at least five subtypes of $\beta 2^*$ nAChRs were found to contribute to striatal dopamine release (Fig. 4a). The $\alpha 4\alpha 6\beta 2\beta 3$ subtype is deduced to have the highest sensitivity (EC_{50} for nicotine-evoked [^3H]dopamine release was 230 nM) (Salminen et al., 2004, 2007). The presynaptic nicotinic modulation of dopamine release demonstrated by using radiolabeled transmitter has been corroborated for endogenous dopamine using fast-scan cyclic voltammetry (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004; Exley et al., 2008; Meyer et al., 2008; Perez et al., 2008).

Superfusion of isolated nerve terminals (that precludes neurochemical cross talk between boutons) has generally failed to demonstrate any contribution from $\alpha 7$ nAChRs that are considered to be absent from these terminals. However, in rat striatal slice preparations some local anatomical integrity is preserved that permits neurochemical cross talk, $\alpha 7$ nAChRs have been shown to enhance [^3H]dopamine release (Kaiser and Wonnacott, 2000). This action is absent in striatal tissue from $\alpha 7$ knockout mice (Quarta et al., 2009) and is blocked by inhibitors of ionotropic glutamate receptors, consistent with the localization of $\alpha 7$ nAChRs on glutamate afferents (Kaiser and Wonnacott, 2000; Marchi et al., 2002; Livingstone and Wonnacott, 2009). There are elaborate reciprocal interactions between dopamine and glutamate afferents in the striatum (Calabresi et al., 1998; Avshalumov et al., 2008). However, no contribution from $\alpha 7$ nAChRs was detected in measurements of endogenous dopamine release evoked by low- or high-frequency stimulation in mouse striatal slices (Zhou et al., 2001; Exley and Cragg, 2008).

Presynaptic $\beta 2^*$ nAChRs seem to act by depolarizing the terminal bouton. This leads to activation of voltage-operated Ca^{2+} channels and influx of Ca^{2+} (in addition to some Ca^{2+} entry through the nAChR channels), and do-

pamine release occurs by classic exocytosis (Soliakov and Wonnacott, 1997; Kulak et al., 2001). The voltage dependence and inward rectification of neuronal nAChRs means that these receptors generate the biggest responses at resting or hyperpolarized membrane potentials (Mulle et al., 1992). Given that dopamine neurons are tonically active, the responsiveness of nAChRs during this pacemaker activity may be compromised. However, local application of nicotine into the striatum of conscious freely moving rats provokes dopamine overflow, consistent with the ability of presynaptic nAChRs to exert a positive effect *in vivo* (Toth et al., 1992; Marshall et al., 1997). Nevertheless, it is clear from the previous section that midbrain nAChRs drive burst firing, and under bursting conditions (which result in greater terminal depolarization), it may be anticipated that presynaptic nAChRs will show diminished responsiveness to agonist.

Most of the *in vitro* studies cited so far have focused on the actions of exogenous agonists, notably nicotine, on pharmacologically defined nAChR subtypes in the striatum, with the goal of exploring the actions of psychomotor stimulants. However, in the context of understanding Parkinson's disease, it is important to comprehend the physiological *modus operandi* of nAChRs. In the striatum, nAChRs will respond to ACh released from cholinergic interneurons; these are tonically active when dopamine neurons are quiescent (tonic firing) but shut down when dopamine neurons switch to burst firing mode (Cragg, 2006). To better understand the physiological impact of presynaptic nicotinic modulation, dopamine release has been explored in slice preparations in which the endogenous firing patterns of dopamine neurons can be simulated by electrical stimulation (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004; Exley et al., 2008; Meyer et al., 2008; Perez et al., 2008). In these studies, detection of dopamine using carbon fiber microelectrodes and fast-scan cyclic voltammetry combines sensitivity with high temporal and spatial resolution. At low-frequency stimulation, dopamine release as a result of the activation of presynaptic nAChRs by endogenous ACh was indicated by the inhibitory effects of vesamicol, to deplete ACh stores, or nAChR antagonists (Zhou et al., 2001). The decreased amounts of dopamine found in response to ambenonium, an acetylcholinesterase inhibitor, were presumed to reflect nAChR desensitization by the sustained concentration of ACh under these conditions. In agreement with this model, bath-applied agonist (including ACh) decreased evoked release. This distinction is important: it emphasizes that although the cholinergic interneurons are tonically active, the frequency of release events is such that ACh signals are terminated by acetylcholinesterase to create a pulsatile delivery, thus avoiding receptor desensitization. This profile is not mimicked by drug application, either experimentally or therapeutically.

The observation by Zhou et al. (2001) that under conditions of low frequency stimulation, nicotine seems to act like a blocker has been confirmed and extended by others

(Rice and Cragg, 2004; Zhang and Sulzer, 2004; Exley and Cragg, 2008). Comparison of the effects of nicotine or mecamylamine at a range of stimulation frequencies revealed that although both drugs suppressed release at tonic frequencies (<10 Hz), they enhanced release by phasic bursts (>25 Hz) (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Exley and Cragg, 2008). Similar effects were reported for primate striatal slices (Perez et al., 2009). Thus, inhibition of nAChRs (by desensitization or antagonist) serves to enhance the contrast between levels of dopamine evoked by phasic versus tonic stimulation. This unexpected result has been explained by the occurrence of use-dependent, short-term depression of dopamine release probability at rapidly successive pulses (Cragg, 2003; Rice and Cragg, 2004; Zhang and Sulzer, 2004). At low-frequency stimulation, endogenous ACh increases release probability by activating nAChRs. However, this leads to short-term depression and less release per pulse in response to high-frequency stimulation (bursts). Reducing nAChR activation (by desensitization or antagonist) decreases short-term depression, permitting a greater response to emerge at higher frequencies. What is missing from this picture is the endogenous behavior under bursting conditions, when cholinergic interneurons are coordinately regulated to cease firing (referred to as a "pause"). Thus, ACh will not be released and nAChRs will not be activated. The consequent decrease in nicotinic stimulation is also predicted to decrease short-term depression and enhance the contrast between the two firing patterns. This mechanism has been referred to as a "cholinergic filter" (Rice and Cragg, 2004; Zhang and Sulzer, 2004) that may represent a unique nicotinic mechanism, because inhibiting dopamine autoreceptors or transporters failed to alter the ratio of phasic/tonic dopamine responses despite increasing dopamine release (Zhang et al., 2009a). A potential limitation of this *in vitro* model is that it is presently uncertain how cholinergic activity changes in response to manipulation of firing patterns experimentally in slice preparations and whether this replicates the "pause" observed *in vivo*.

The $\beta 2$ -selective antagonist dihydro- β -erythroidine mimics the effects seen with mecamylamine, confirming that $\beta 2^*$ nAChRs account for this phenomenon (Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004). The contribution of the $\alpha 6\beta 2^*$ nAChR subpopulation was dissected using the $\alpha 6\beta 2^*$ -selective antagonist α -conotoxinMIII (Exley et al., 2008; Meyer et al., 2008; Perez et al., 2008). In mouse, $\alpha 6\beta 2^*$ and $\alpha 4(\text{non-}\alpha 6)\beta 2^*$ nAChRs were distinguishable by their sensitivity to stimulus intensity, responding to low and high stimulus strengths, respectively. This result prompted the speculation that these nAChR subtypes may be localized to separate dopaminergic fibers, although it is possible they are expressed on the same fibers but have different sensitivity to acetylcholine such that weak stimulation elicits less acetylcholine release than strong stimulation (Meyer et al., 2008). The contributions of $\alpha 4(\text{non-}\alpha 6)\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs were

found to vary with experimental parameters, the latter appearing to have a lesser impact under phasic stimulation conditions (Exley et al., 2008; Meyer et al., 2008; Perez et al., 2008). Comparison of responses across the mouse striatum indicated that $\alpha 6\beta 2^*$ nAChRs exerted a more prominent effect on the nicotinic “filter” that modulates dopamine release in nucleus accumbens (ventral striatum) than in caudate putamen (dorsal striatum) (Exley et al., 2008). In primates, $\alpha 6/\alpha 3\beta 2^*$ nAChRs seemed equally effective in both striatal regions when dopamine release was evoked by a single stimulus, accounting for at least 80% of the nicotinic modulation (Perez et al., 2009). In contrast, when stimulation was delivered as a train of four pulses to simulate a burst, dopamine release with $\alpha 6/\alpha 3\beta 2^*$ nAChR blockade was overcome in the dorsal but not ventral striatum from monkeys (Perez et al., 2009). These data demonstrate that $\alpha 6\beta 2^*$ nAChRs differentially control dopamine release in these two regions.

In a comparison of dorsal and ventral striatum, and the nucleus accumbens shell, Zhang et al. (2009b) showed that the dorsal striatum displays a higher probability of release in response to a single stimulus, representing tonic (low-frequency) stimulation in both primates (Cragg, 2003) and rodents (Zhang et al., 2009b). Consequently, this region showed less frequency-dependent facilitation (determined as the ratio of responses to 20 pulses/1 pulse, delivered at 20 Hz). Blockade of $\beta 2^*$ nAChRs, dopamine transporters or D2 receptors indicated that nAChRs are primarily responsible for the differential frequency dependence of dopamine neurons in the dorsal striatum. Whereas these studies (Zhang et al., 2009b) did not consider the contribution of particular $\beta 2^*$ nAChR subtypes, Drenan et al. (2010) found that a gain-of-function mutation in the $\alpha 6$ nAChR subunit changed the pattern of stimulus-dependent dopamine release to one that more closely resembled that found in ventral striatum. The mutant mice showed reduced synaptic depression and increased frequency-dependent facilitation (the ratio of responses to four pulses/one pulse, delivered at 100 Hz) in dorsal striatum slices, compared with results from wild-type mice. Moreover, the kinetics of the dopamine waveforms were altered. One interpretation of this study is that presynaptic $\alpha 6\alpha 4\beta 2^*$ nAChRs have a major role in locally shaping dopamine release in the striatum, but alternative explanations are possible, and it should be remembered that the gain of function mutation creates a nAChR with considerably altered activity. Indeed, many factors could account for these observed changes, including adaptive alterations during development of the mutant animals.

The impact of $\alpha 7$ nAChRs (presumed to reside on glutamate afferents to the striatum) on nicotinic filtering of dopamine release has received less attention. Antagonism of $\alpha 7$ nAChRs had no effect on dopamine release in response to low- or high-frequency stimulation of mouse striatal slices (Zhou et al., 2001), but recent studies in rat striatal slices revealed a subtle contribution of $\alpha 7$ nAChRs to nicotinic modulation at high-frequency stimulation (Sei-

pel and Yakel, 2010). The integration of multiple transmitter influences (including GABA, glutamate, and serotonin) that may be modulated by presynaptic nAChRs and their local impact on dopamine release is not yet understood.

In summary, although the firing pattern exhibited by dopamine neurons is the major determinant of dopamine release probability in the striatum, evidence is emerging that the multiplicity of heteromeric nAChR subtypes on dopamine terminals is important for locally shaping dopamine responses. Presynaptic nAChRs are proposed to act as filters to interpret dynamic ACh signals that are reciprocally coordinated with dopamine neuron firing. Thus nAChRs serve to discriminate tonic and phasic patterns of stimulation. Nicotinic drugs can amplify this discrimination, but they do this by nAChR desensitization or inhibition, such that nicotinic agonists achieve the same effect as antagonists. Both $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs contribute to the regulation of striatal dopamine release, with the $\alpha 6\beta 2^*$ nAChR population playing a dominant role in the nucleus accumbens and also making a significant contribution in the striatum. These observations have implications for the development of drugs with optimal benefit for Parkinson's disease.

C. Downstream Dopaminergic Signaling Mechanisms Linked to Nicotinic Acetylcholine Receptors

The concept that different populations of striatal medium spiny projection neurons are responsible for distinct aspects of motor control resulted in the classification of the “direct” and “indirect” pathways (Albin et al., 1989; Graybiel et al., 1990; Parent, 1990) (Fig. 3). These circuits act in an opposing fashion with the direct pathway, resulting in disinhibition of the thalamus or brain stem nuclei, whereas the indirect pathway exerts an inhibitory influence. The nigrostriatal dopaminergic inputs differentially regulate these two pathways through the segregation of dopamine D1 and D2 receptors to medium spiny neurons of the direct and indirect pathways, respectively (Gerfen et al., 1990). Thus, in simplistic terms, dopamine acting via stimulatory D1 receptors serves to enhance disinhibition, whereas its actions through inhibitory D2 receptors decrease inhibition. The net effect is the relief of the brake on thalamocortical drive to the motor cortex, favoring the initiation or smooth execution of motor function. This is consistent with the bradykinesia characteristic of Parkinson's disease when the ‘permissive’ nigrostriatal projection degenerates. Therapies predicted to boost dopamine release in the striatum, for example agonists targeting $\alpha 4\beta 2^*$ or $\alpha 6\beta 2^*$ nAChRs, would help to counteract the “brake.” Therefore, it is of interest to understand the impact of nAChR regulation on downstream dopamine receptor-linked mechanisms within the postsynaptic neurons.

In medium spiny neurons of the striatum an important site of signal integration that is potentially modulated by nAChRs is the protein DARPP-32. Multiple signaling pathways converge to regulate this “molecular switch,” identified as dopamine and cAMP regulated phosphopro-

tein with molecular weight 32 kDa (DARPP-32) (Svenningsson et al., 2004) (Fig. 5). The activity of DARPP-32 is promoted or reduced by dopamine acting via D1 or D2 receptors, respectively. DARPP-32 is expressed in both striatonigral (“direct”) and striatopallidal (“indirect”) neurons and its selective deletion in these subsets of neurons results in decreased and increased locomotor function, respectively. This is consistent with the proposed roles of the direct and indirect pathways and establishes a contribution of DARPP-32 signaling to this regulation (Bateup et al., 2010).

DARPP-32 is a key determinant of medium spiny neuron excitability by virtue of its ability to regulate the phosphorylation status of various targets (including receptors, ion channels, and transcription factors) through the inhibition of the multifunctional protein phosphatase 1 (PP-1). D1 receptor stimulation is reported to increase currents through α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors and L-type Ca^{2+} channels in striatal neurons, and this depends upon DARPP-32 inhibition of PP-1 (Surmeier et al., 1995; Yan et al., 1999). DARPP-32 knockout mice lack the ability to produce D1 receptor-mediated potentiation of NMDA-evoked currents (Flores-Hernández et al., 2002). Selective deletion of DARPP-32 in striatonigral or striatopallidal neurons results in functional deficits in long-term potentiation in both neuronal populations (Bateup et al., 2010).

nAChR stimulation is predicted to influence the excitability of postsynaptic striatal neurons via increases in dopamine release (Fig. 5). Indeed, *in vivo* administration of

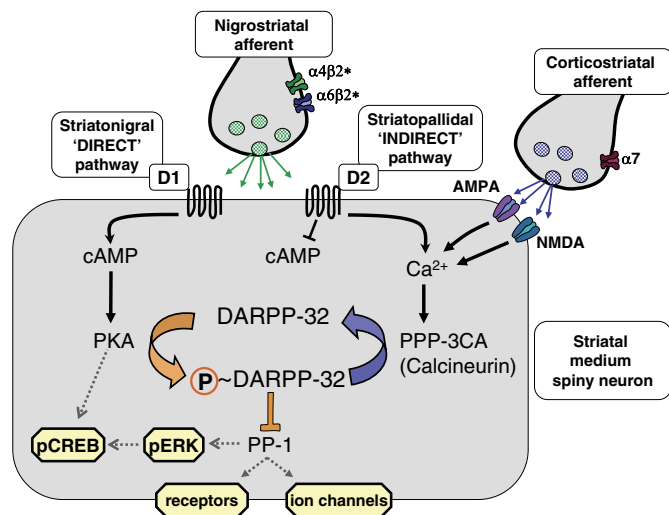


FIG. 5. Pivotal role of DARPP-32 in postsynaptic signaling in medium spiny neurons, illustrating the potential for nicotinic modulation. DARPP-32 integrates inputs from multiple systems to regulate PP-1; only dopamine and glutamate receptors are shown for clarity. Postsynaptic dopamine D1 and D2 receptors are largely segregated to the “direct” (striatonigral) and “indirect” (striatopallidal) projection pathways, respectively. Other components shown are presumed to be common to all medium spiny neurons. DARPP-32 via PP-1 influences the activity of numerous target proteins, including receptors, ion channels and transcription factors. nAChRs that modulate dopamine release, notably $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ subtypes (shown here on nigrostriatal terminals but also present on dopaminergic cell bodies in the SN) can also affect postsynaptic excitability and synaptic plasticity through these mechanisms.

psychomotor stimulants, including nicotine, that promote dopamine release have been shown to increase DARPP-32 phosphorylation and activation of extracellular signal-regulated kinase in a subset of striatal medium spiny neurons (Valjent et al., 2005). Nicotine increased the phosphorylation of striatal DARPP-32 at multiple sites in mice given systemic injections of nicotine (Zhu et al., 2005). In rat striatal slices *in vitro*, nicotine has been reported to modify DARPP-32 phosphorylation in a concentration- and time-dependent manner, by acting at $\beta 2^*$ and $\alpha 7$ nAChRs (Hamada et al., 2004; Hamada et al., 2005). Sensitivity to dopamine receptor antagonists supports the view that nicotine-evoked changes in DARPP-32 phosphorylation depend on dopamine release. It is unclear whether the same or different changes occur in striatonigral and striatopallidal neurons, although the observation of distinct dopamine D1 and D2 receptor-mediated responses is consistent with their segregation.

The therapeutic efficacy of L-DOPA in Parkinson’s disease relies on the preservation of normal postsynaptic signaling mechanisms. The evidence from animal models of the disease, as well as neurochemical assessment of post mortem human brain tissue from patients, suggests that levels of DARPP-32 are unchanged after nigrostriatal denervation (Raisman-Vozari et al., 1990; Nishino et al., 1993). However, functional changes in DARPP-32 may still ensue; for example, increased phosphorylation of DARPP-32 at the inhibitory Thr75 site (with no change in phosphorylation of the activating Thr34 site, compared with healthy controls) has been reported (Brown et al., 2005). DARPP-32 signaling is also implicated in the development of L-DOPA-induced dyskinesia (Santini et al., 2007) (see section VIII.A below).

In summary, the control of motor function via the balance of activity in the direct and indirect pathways projecting from the striatum is influenced by dopamine acting at dopamine D1 and D2 receptors, respectively. The ability of dopamine to modify activity within the medium spiny neurons is achieved, at least in part, via shifts in the balance of phosphorylation and dephosphorylation manipulated by the key regulator protein DARPP-32, an inhibitor of PP-1. DARPP-32 integrates signals from nigrostriatal and other inputs (notably the corticostriatal afferents that release glutamate onto medium spiny neurons) to regulate excitability and longer lasting functions, including synaptic plasticity in the projection neurons. Nicotinic stimulation (studies to date have mostly focused on nicotine) can influence DARPP-32 signaling, predominantly by promoting dopamine release via $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs, although $\alpha 7$ nAChR-mediated glutamate release has also been implicated *in vitro*, at higher nicotine concentrations (Hamada et al., 2004). More studies are needed to elaborate the effects of selective nAChR activation on postsynaptic molecular mechanisms. The ability of nAChRs to influence signaling within the direct and indirect pathways is compatible with their therapeutic potential for treating Parkinson’s disease.

IV. Long-Term Regulation of Nicotinic Acetylcholine Receptors Expression and Function

A. Effect of Long-Term Nicotine Administration

Before nicotine can be used therapeutically for neurodegenerative disorders, it is important to understand the long-term consequences of its administration on nAChR subtype expression and function. As mentioned earlier, short-term exposure results in numerous biological responses because nicotine acts in diverse tissues throughout the body and interacts with different nAChR subtypes at a given site. Repeated administration adds an additional layer of complexity because of receptor desensitization, tolerance, sensitization, up-regulation, and down-regulation. (Corringer et al., 2006; Quik and McIntosh, 2006; Picciotto et al., 2008; Walsh et al., 2008; Buccafusco et al., 2009). A comprehensive knowledge of these changes is important for a clear understanding of the mechanisms whereby nicotine protects against nigrostriatal damage and/or improves L-DOPA-induced dyskinesias.

As discussed earlier, one of the most widely distributed CNS nAChRs is the $\alpha 4\beta 2^*$ subtype. Long-term nicotine administration, delivered in multiple forms (injection, minipump, drinking water, or self-administration), increases the numbers of $\alpha 4\beta 2^*$ binding sites throughout the brain in experimental animal models and humans (Marks et al., 1983; Schwartz and Kellar, 1983; Benwell et al., 1988; Perry et al., 1999; Staley et al., 2006; Xiao et al., 2009; Moretti et al., 2010). There was some initial controversy concerning $\alpha 4\beta 2^*$ receptor up-regulation in the striatum, because increases were observed in some studies but not others. This discrepancy now seems to have arisen because the radioligands originally used for receptor identification bound to multiple nAChR subtypes that were differentially up- and down-regulated with nicotine treatment. This problem has now been circumvented through the use of α -conotoxinMII, which binds to $\alpha 6\beta 2^*$ nAChRs. It thus allows for the selective identification of $\alpha 4\beta 2^*$ receptor subtypes by a subtractive process. Studies with α -conotoxinMII show that striatal $\alpha 4\beta 2^*$ receptors are indeed up-regulated by nicotine treatment. Current evidence suggests that two $\alpha 4\beta 2^*$ nAChR populations exist in striatum (i.e., the $\alpha 4\beta 2$ and the $\alpha 4\alpha 5\beta 2$ subtypes) (Grady et al.,

2010b; Moretti et al., 2010) (Fig. 4a). These are differentially regulated by nicotine, with an increase in the $\alpha 4\beta 2$ subtype but no change in the $\alpha 4\alpha 5\beta 2$ receptor (Table 1) (Moretti et al., 2010). These findings underscore the complex changes that arise in $\alpha 4\beta 2^*$ receptor-mediated responsiveness with drug treatment.

Nicotine exposure differentially controls $\alpha 6\beta 2^*$ nAChR subtype expression compared with that of $\alpha 4\beta 2^*$. Nicotine administration for several days either decreases or does not change striatal $\alpha 6\beta 2^*$ nAChR levels (Nguyen et al., 2003; Lai et al., 2005; McCallum et al., 2006; Mugnaini et al., 2006; Perry et al., 2007; Moretti et al., 2010). To add to the complexity, the $\alpha 6\beta 2^*$ nAChR subtypes present in striatum (Fig. 4a) are themselves differently affected by nicotine treatment. Studies with the novel α -conotoxinMII analog E11A, which discriminates between $\alpha 4\alpha 6\beta 2\beta 3$ and $\alpha 6\beta 2\beta 3$ subtypes, show that nicotine treatment selectively decreased the former but increased the latter population in mouse striatum (Perez et al., 2008). This up-regulation of $\alpha 6\beta 2\beta 3$ nAChRs is in agreement with expression studies in culture that also show that $\alpha 6\beta 2$ or $\alpha 6\beta 2\beta 3$ receptors are up-regulated with long-term nicotine exposure (Tumkosit et al., 2006; Walsh et al., 2008). It should be noted, however, that these differential nicotine-induced changes in mouse $\alpha 6\alpha 4\beta 2\beta 3$ and $\alpha 6\beta 2\beta 3$ subtypes did not occur in rat striatum, suggesting it may be species-dependent or possibly related to differences in treatment paradigms between rats and mice (Perez et al., 2008; Huang et al., 2009).

Another nAChR present in striatum is the $\alpha 7$ subtype (Fig. 4a). Its functional role is less clear because studies are confounded by the very low expression of $\alpha 7$ nAChRs in rat, monkey, and human striatum. However, it is readily detected in mouse striatum, where it may be increased or not affected by nicotine administration (Pauly et al., 1991; Lai et al., 2005; Moretti et al., 2010). These variable results may reflect a requirement for higher concentrations of nicotine to up-regulate $\alpha 7$ nAChRs compared with $\alpha 4\beta 2^*$ nAChRs.

The molecular basis for the differential regulation of various striatal nAChR subtypes is an area actively under investigation, the $\alpha 4\beta 2^*$ nAChR subtype being most extensively investigated to date (Govind et al., 2009; Lester et

TABLE 1
Alterations in striatal nAChR subtype expression with chronic nicotine treatment

nAChR Subtype	Effect of Nicotine Treatment	System	References
$\alpha 6\beta 2^*$	↓ or No change	Rats, mice, monkeys	Lai et al., 2005; Mugnaini et al., 2006; Perry et al., 2007; Moretti et al., 2010; but see Parker et al., 2004
$\alpha 6\alpha 4\beta 2\beta 3$	↓	Rats, mice	Perez et al., 2008; Huang et al., 2009
$\alpha 6\beta 2\beta 3$	↑	Mice, cultured cells	Tumkosit et al., 2006; Perez et al., 2008; Walsh et al., 2008
$\alpha 6\beta 2\beta 3$	↓	Rats	Huang et al., 2009
$\alpha 3\beta 2$	No change	Rats	Moretti et al., 2010
$\alpha 4\beta 2$	↑	Rats, mice, monkeys	Marks et al., 1983; Schwartz and Kellar, 1983; Benwell et al., 1988; Perry et al., 1999; McCallum et al., 2006; Staley et al., 2006; Xiao et al., 2009; Moretti et al., 2010
$\alpha 4\alpha 5\beta 2$	No change	Rats	Moretti et al., 2010
$\alpha 7$	↑ or No change	Rats, mice, monkeys	Pauly et al., 1991; Quik et al., 2000a; Lai et al., 2005; Moretti et al., 2010

al., 2009). There is a consensus that up-regulation of $\alpha 4\beta 2^*$ nAChRs is primarily controlled at the post-transcriptional level, with little change in $\alpha 4$ or $\beta 2$ nAChR mRNA levels (Marks et al., 1992). Various mechanisms have been proposed to explain nicotine-mediated up-regulation (Picciotto et al., 2008; Buccafusco et al., 2009; Benowitz, 2010; Changeux, 2010b; Mao and McGehee, 2010). One hypothesis is that the initial receptor desensitization contributes to up-regulation, possibly via receptor phosphorylation (Léna and Changeux, 1993; Swope et al., 1999; Wiesner and Fuhrer, 2006). More recently, it has been proposed that nicotine acts as a chaperone to promote maturation of $\alpha 4$ nAChR subunit precursors that might otherwise be degraded by currently unidentified endogenous molecular components (Salette et al., 2005; Corringer et al., 2006; Tumkosit et al., 2006; Kuryatov et al., 2008; Walsh et al., 2008; Lester et al., 2009; Jones et al., 2010). The enhanced incorporation of $\alpha 4$ subunits into the $\alpha 4\beta 2$ nAChR may subsequently influence agonist-mediated regulation of other nAChR subtypes. For instance, the enhanced formation of $\alpha 4\beta 2$ receptors may reduce free $\alpha 4$ subunit levels, resulting in a decline in the $\alpha 6\alpha 4\beta 2\beta 3$ receptor subtype. By contrast, $\alpha 6\beta 2\beta 3$ receptor levels may still increase because they do not require the $\alpha 4$ subunit. The presence of the $\beta 3$ subunit in $\alpha 6\beta 2^*$ nAChRs also increases receptor up-regulation (Tumkosit et al., 2006). Receptor up- and down-regulation is not only controlled by the nature of the subunits in the nAChR complex but is also influenced by chaperone proteins, such as RIC-3, neurexin1 β , VLIP-1, and others (Wanamaker and Green, 2007; Millar, 2008; Cheng et al., 2009; Zhao et al., 2009). As well, work suggests that the ubiquitin-proteasome system regulates the stability of neuronal nAChRs with a resultant receptor up-regulation (Rezvani et al., 2010). Numerous mechanisms thus exist to modulate receptor expression and responsiveness with long-term nAChR drug administration.

A balance between these differing molecular mechanisms would ultimately determine the short- and long-term consequences of nicotinic drugs on nAChR subtype expression. This in turn would control nAChR-mediated behaviors linked to Parkinson's disease, such as locomotor activity.

B. Effect of Nigrostriatal Damage

As briefly indicated (section III.A.3), damage to the nigrostriatal dopaminergic pathway has a major impact on nAChR expression in striatum in parkinsonian animal models (Schwartz et al., 1984; Quik et al., 2001, 2003; Kulak et al., 2002a; Zoli et al., 2002; Champtiaux et al., 2003) and in Parkinson's disease cases (Aubert et al., 1992; Perry et al., 1995; Court et al., 2000; Quik et al., 2004; Bohr et al., 2005; Gotti et al., 2006a). There seems to be a particularly pronounced decline in $\alpha 6\beta 2^*$ nAChRs, losses paralleling those in the dopamine transporter, a well established marker of striatal dopaminergic terminals (Table 2) (Quik et al., 2001, 2003; Zoli et al., 2002). These data provide one of the pieces of evidence that $\alpha 6\beta 2^*$ nAChR are primarily located on incoming dopaminergic afferents to the striatum (Fig. 4a). Further work done with α -conotoxin E11A, a toxin that discriminates between $\alpha 6\beta 2^*$ nAChR subtypes, showed that the $\alpha 4\alpha 6\beta 2\beta 3$ receptor is the first to be lost with nigrostriatal damage, with decreases in the $\alpha 6\beta 2\beta 3$ receptor subtype only with more severe lesioning (Bordia et al., 2007). This pattern of decline is observed across species (mice, rats, monkeys, humans), highlighting the possibility that the $\alpha 4\alpha 6\beta 2\beta 3$ receptor subtype may identify a particularly vulnerable population of dopaminergic afferents to the striatum.

By contrast to the dramatic declines in $\alpha 6\beta 2^*$ nAChRs with nigrostriatal damage, the decrease in the $\alpha 4\beta 2^*$ nAChR population that does not include $\alpha 6$ is much less severe (Table 2). The reason for the apparently smaller decline in the $\alpha 4\beta 2^*$ receptor subtype most likely relates to its more diverse localization, with receptors present on dopamine and GABAergic terminals and possibly on some GABAergic interneurons and serotonin afferents in the striatum (Kulak et al., 2002a; Zoli et al., 2002; Champtiaux et al., 2003; Quik et al., 2003, 2004; Bohr et al., 2005) (see section.III.A.2; Fig. 4a). An almost complete dopaminergic lesion leads to only a 30 to 50% decline in striatal $\alpha 4\beta 2^*$ nAChRs; this would suggest that 50 to 70% of the $\alpha 4\beta 2^*$ receptors are present on dopaminergic terminals (Kulak et al., 2002a; Zoli et al., 2002; Champtiaux et al., 2003; Quik et al., 2003). Studies using synaptosomal preparations, which primarily contain nerve terminals, suggest that stri-

TABLE 2
Decline in nAChR subtype expression with nigrostriatal damage

Moderate lesion is a $\geq 80\%$ decline in the dopamine transporter; a severe lesion is $\geq 95\%$ decline in the dopamine transporter.

nAChR subtype	Present on Striatal Dopamine Terminals	Severity of Lesion		Reference
		Moderate	Severe	
$\alpha 6\beta 2^*$	Yes	↓↓	↓↓↓	Quik et al., 2001, 2005; Zoli et al., 2002
$\alpha 6\alpha 4\beta 2\beta 3$	Yes	↓↓	↓↓↓	Bordia et al., 2007
$\alpha 6\beta 2\beta 3$	Yes	—	↓↓↓	Bordia et al., 2007
$\alpha 3\beta 2$	Yes	N.D.	↓↓↓	Quik et al., 2005
$\alpha 4\beta 2$	Yes	↓	↓↓	Zoli et al., 2002; Quik et al., 2005
$\alpha 4\alpha 5\beta 2$	Yes	↓	↓↓	Zoli et al., 2002
$\alpha 4\beta 2^*$	No	—	—	
$\alpha 7$	No	—	—	Zoli et al., 2002; Quik et al., 2005

↓, decline; —, no change; N.D., not determined.

atal presynaptic $\alpha 4\beta 2^*$ nAChRs consist of both the $\alpha 4\beta 2$ and the $\alpha 4\alpha 5\beta 2$ subtypes and are present on dopaminergic as well as other neurons (McClure-Begley et al., 2009; Grady et al., 2010b). Further evidence for this stems from studies showing that the $\alpha 5$ subunit declines by $\sim 90\%$ with nigrostriatal damage whereas the $\alpha 4$ subunit is decreased by only $\sim 50\%$ (Zoli et al., 2002). These data suggest that the greater proportion of $\alpha 4\alpha 5\beta 2$ nAChRs are present on dopaminergic terminals in the striatum, whereas the $\alpha 4\beta 2$ receptors are distributed on various neuronal types (Fig. 4a). Thus, with near complete denervation, there would be a major loss of the $\alpha 4\alpha 5\beta 2$ but only a partial decline in the $\alpha 4\beta 2$ receptor subtype.

Studies to evaluate effects of nigrostriatal damage on striatal $\alpha 7$ nAChRs are difficult because of their low expression levels in rat, monkey, and human striatum. However, where such studies have been done, $\alpha 7$ receptor expression seems not to change with lesioning (Quik et al., 2000a, 2003). These findings are consistent with the concept that $\alpha 7$ nAChRs are present on nondopaminergic terminals, including notably glutamatergic afferents, in the striatum (Fig. 4a) (Kaiser and Wonnacott, 2000).

Although there has been a general focus on the striatum, it is also important to consider the fate of nAChRs in the SN pars compacta with nigrostriatal damage. Work in this brain region is much more limited; however, studies in rats and monkeys have shown that nAChR binding is decreased with lesioning (Clarke and Pert, 1985; Quik et al., 2002, 2010). With respect to subtype selectivity, there is a more pronounced decline in $\alpha 6\beta 2^*$ compared with $\alpha 4\beta 2^*$ nAChR expression in the SN pars compacta with nigrostriatal damage (Quik et al., 2002, 2010). Because the decrease in $\alpha 6\beta 2^*$ nAChRs correlates with that in the dopa-

mine transporter, these data are consistent with the localization of $\alpha 6\beta 2^*$ nAChR to somatodendritic sites on dopaminergic neurons (Klink et al., 2001). By contrast, $\alpha 4\beta 2^*$ nAChRs are present on dopaminergic neurons and GABAergic interneurons and afferents from the SN pars reticulata. Thus, residual somatodendritic nAChRs left after partial lesions or in the early stages of Parkinson's disease also offer credible therapeutic targets for nicotinic drugs, especially in view of their importance in driving burst firing and striatal dopamine release (see section III B.1). This identifies another area that merits further study.

To conclude, the nAChRs most affected by nigrostriatal damage are those present on dopaminergic neurons in the nigrostriatal pathway. The most pronounced losses are observed in the $\alpha 6\beta 2^*$ and $\alpha 4\alpha 5\beta 2$ subtypes; smaller declines are found in $\alpha 4\beta 2$ receptors, and there is no change in the $\alpha 7$ receptor subtype. These data, summarized in Fig. 6, suggest that drugs that target $\alpha 6\beta 2^*$ and $\alpha 4\beta 2^*$ nAChRs may prove most useful in ameliorating nAChR-mediated function with nigrostriatal damage.

V. Role for Nicotine and Nicotinic Acetylcholine Receptor Ligands in Parkinson's Disease

Parkinson's disease is frequently considered a disorder with a primary dysfunction of the nigrostriatal dopaminergic pathway. However, as discussed, the dopaminergic system is integrated with numerous other CNS systems, including the nicotinic cholinergic system (Calabresi and Di Filippo, 2008; Exley and Cragg, 2008; Barik and Wonnacott, 2009). The close functional interaction between the cholinergic and dopaminergic systems forms the basis for

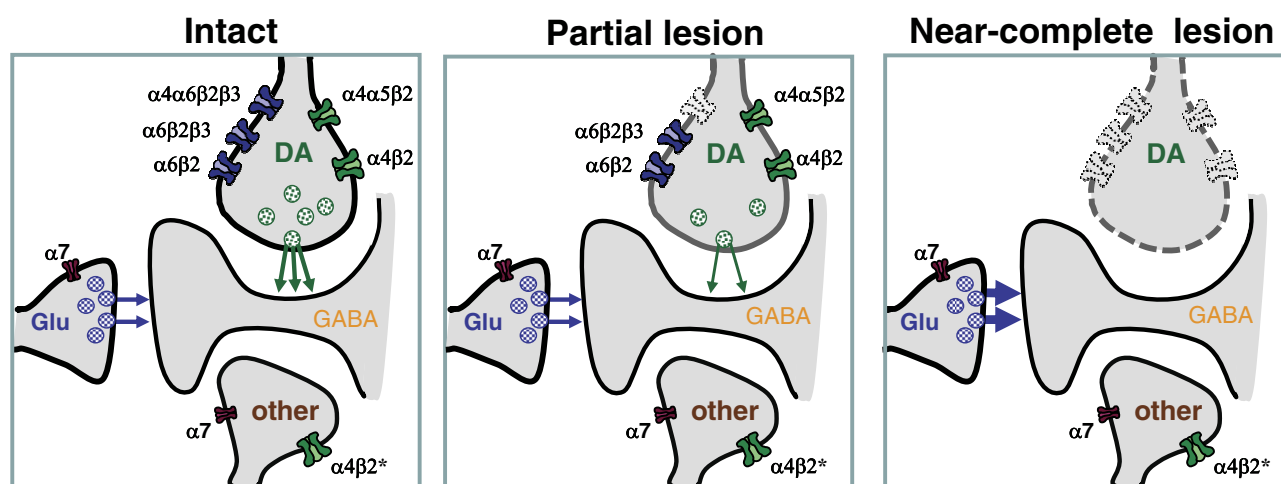


FIG. 6. Schematic diagram depicting the effects of partial and near-complete dopaminergic lesioning on expression of nAChR subtypes in striatum. In intact striatum (left), $\alpha 6\beta 2^*$ nAChRs are primarily expressed on nigrostriatal dopaminergic terminals (DA). $\alpha 4\beta 2^*$ nAChRs are also located on dopaminergic terminals and comprise 30 to 50% of the striatal $\alpha 4\beta 2^*$ population. The remaining 70 to 50% of $\alpha 4\beta 2^*$ nAChRs, which are likely to be predominantly composed only of the $\alpha 4$ and $\beta 2$ subunits, are present on other neuronal or non-neuronal elements (other). $\alpha 7$ nAChRs are inferred to be located on glutamatergic (Glu) afferents. In rats with a partial striatal lesion (middle), the $\alpha 6\alpha 4\beta 2\beta 3$ nAChR subtype disappears first, and thus the effects of nicotine or nicotinic drugs would be mediated by residual $\alpha 6\beta 2^*$ subtypes and by $\alpha 4\beta 2^*$ and possibly $\alpha 7$ nAChR subtypes. With a near-complete lesion (right), all presynaptic nAChRs are lost from nigrostriatal dopaminergic terminals. However, $\alpha 4\beta 2$ nAChRs are preserved on other elements and, possibly together with $\alpha 7$ nAChRs, will contribute to local nAChR-mediated effects. Enhanced glutamatergic signaling is indicated. See Huang et al. (2011) for further details.

the hypothesis that nAChR drugs may be useful for Parkinson's disease therapy. Accumulating evidence suggests that nicotine and nAChR ligands may influence Parkinson's disease motor symptoms via two distinct mechanisms: 1) they may protect against nigrostriatal damage to improve motor control over the long term. 2) In addition, nAChR ligands may directly stimulate the dopaminergic system to acutely ameliorate motor-related symptoms.

In the following sections, we describe the evidence that nAChR drugs may be valuable as a disease-modifying therapy for neuroprotection against nigrostriatal degeneration and may also be helpful for alleviating treatment-related side effects, specifically L-DOPA-induced dyskinesias.

VI. Smoking, Nicotine, and Neuroprotection against Nigrostriatal Damage

A. Epidemiological Studies and Smoking

Over a half-century of studies show overwhelmingly that smoking is inversely associated with Parkinson's disease (Morens et al., 1995; Gorell et al., 1999; Hernán et al., 2001; Allam et al., 2004; Alves et al., 2004; Ritz et al., 2007; Thacker et al., 2007; Elbaz and Moisan, 2008; Morozova et al., 2008; Chen et al., 2010; Ritz and Rhodes, 2010) and other Lewy body-related pathologic conditions (Tsuang et al., 2010). A prime question is whether this decreased incidence of Parkinson's disease in those with a history of smoking is due to the increased mortality risk associated with smoking. This appears not to be the case as data from several epidemiological studies, including a large prospective cohort, showed that mortality in patients with Parkinson's disease was not influenced by smoking status; that is, mortality was similar in nonsmoking and smoking patients with Parkinson's disease (Alves et al., 2004; Driver et al., 2008). The inverse correlation between smoking and Parkinson's disease is dose-dependent, with a decreased disease incidence with both increased smoking intensity (number of cigarettes smoked per day) and number of years of smoking (Morens et al., 1995; Gorell et al., 1999; Hernán et al., 2001; Allam et al., 2004; Alves et al., 2004; Ritz et al., 2007; Thacker et al., 2007; Elbaz and Moisan, 2008; Morozova et al., 2008; Chen et al., 2010; Ritz and Rhodes, 2010). The reduced Parkinson's disease incidence seems to be independent of sex and education, is more pronounced in current than in former smokers, and also occurs with other forms of tobacco, such as cigars, pipes, chewing tobacco, or snuff (O'Reilly et al., 2005; Ritz et al., 2007).

The findings that the lower risk of Parkinson's disease is inversely correlated with smoking duration, intensity, and recentness but is not linked to increased mortality suggests a true biologic effect. These data may constitute an important clue in identifying drugs that protect against the neurodegenerative changes in Parkinson's disease. It may be relevant that $\alpha 4\beta 2^*$ nAChRs are up-regulated in current but not former smokers (Breese et al., 1997).

B. Nicotine Neuroprotection in Parkinsonian Animal Models

An important question is the identity of the agent(s) in tobacco that contributes to this putative neuroprotective effect of smoking, because its use may reduce Parkinson's disease progression. This would represent a milestone in Parkinson's disease treatment, because current therapies provide only symptomatic relief. Certainly there are thousands of chemicals in tobacco smoke, any of which may have therapeutic value. These include nicotine and components that inhibit monoamine oxidase; however, there has been a focus on nicotine because it is present in fairly high concentrations in tobacco and stimulates dopamine release, as discussed earlier.

One approach to evaluate a role for nicotine is to investigate its neuroprotective properties against nigrostriatal damage in parkinsonian animal models (Belluardo et al., 2000; O'Neill et al., 2002; Quik et al., 2007b; Picciotto and Zoli, 2008; Bencherif, 2009). A frequently used rat model involves unilateral administration of 6-hydroxydopamine into the striatum, SN pars compacta, or medial forebrain bundle or hemisection of the nigrostriatal dopaminergic pathway (Figs. 1 and 6) (Cenci and Lundblad, 2007). These treatment protocols result in unilateral declines in striatal dopaminergic function, with contralateral deficits in motor activity. Bilateral models are not used because these are incompatible with animal survival (Cenci and Lundblad, 2007). In rats, nicotine pretreatment before lesioning reduces neuronal damage as assessed using markers of striatal dopaminergic integrity, such as dopamine and metabolite levels, tyrosine hydroxylase, the dopamine transporter, and the vesicular monoamine transporter. As expected, the degree of protection against nigrostriatal damage is dependent on lesion size, with optimal effectiveness against moderate lesioning. Nicotine dosing is also an important variable. Nicotine exhibits a U-shaped dose-response curve; that is, maximal protection is seen with intermediate nicotine dosing regimens (Costa et al., 2001; Ryan et al., 2001; Soto-Otero et al., 2002). Studies using MPTP-lesioned monkeys, a model that more closely mimics features of Parkinson's disease, also show that a 2-month nicotine pretreatment period protects against nigrostriatal damage (Quik et al., 2006a,b). As reviewed earlier, the ability of nicotine to protect against dopaminergic damage in parkinsonian mice has been inconsistent with positive results in some studies but not others (Quik et al., 2007b). A likely explanation for this variability in mice may relate to their rapid rate of nicotine metabolism ($t_{1/2} = 5\text{--}10$ min in mice compared with 60 min in rats and monkeys) (Matta et al., 2007).

Although nicotine is clearly protective against toxin-induced nigrostriatal damage in parkinsonian rats and monkeys, it should be noted that nicotine does not restore function if administered to parkinsonian animals when nigrostriatal damage is complete (Huang et al., 2009). A 2- to 3-week nicotine regimen given before nigrostriatal dam-

age improved dopamine transporter levels in the striatum of lesioned rats compared with animals not receiving nicotine. However, there was no change in transporter levels when nicotine was administered to rats after lesioning and subsequently given nicotine for the same time interval (Huang et al., 2009). These observations suggest that nicotine's primary action is protection against ongoing degeneration rather than restoration of damaged neurons.

In addition to nicotine, smoke contains numerous other components, any of which may play a synergistic and/or additive role in protection against nigrostriatal damage. As mentioned earlier, this includes agents that modulate metabolic enzymes such as monoamine oxidase (Castagnoli and Murugesan, 2004). The rationale for this possibility stemmed from studies showing reduced levels of monoamine oxidase activity in the brains of smokers (Fowler et al., 1996a,b). In addition, other work showed that tobacco components that inhibit monoamine amine oxidase activity protected against nigrostriatal damage in mouse striatum (Castagnoli et al., 2001). These data provide support for the idea that monoamine oxidase inhibitors in tobacco may decrease enzymic activity in the brains of patients with Parkinson's disease, thereby reducing the synthesis of endogenous toxins that cause nigrostriatal damage in addition to prolonging the availability of dopamine. Nicotine and/or other agents in tobacco may also act by modifying brain expression of cytochrome P450 (P450). This possibility stemmed from work showing that enzymic activity is higher in smokers and is induced by nicotine in experimental animals. Enhanced P450 expression may contribute to neuroprotection by increasing the breakdown of toxic agents that damage the nigrostriatal dopaminergic system (Miksys and Tyndale, 2006).

In summary, epidemiological data show that smoking represents the largest, most consistent negative risk factor for Parkinson's disease. Experimental studies with parkinsonian animals demonstrate that nicotine significantly reduces nigrostriatal damage, with an average 30% protection in different animal models. These combined data

support the hypothesis that nicotine treatment may yield a therapeutic strategy to reduce Parkinson's disease progression. Motor symptoms generally only arise in Parkinson's disease when nigral dopaminergic neurons are reduced by >50% and striatal dopamine by >70% (Olanow, 2004; Samii et al., 2004). Therefore, a 30% protection against nigrostriatal damage may allow for normal function for many years before Parkinson's disease symptoms arise. Although studies have been done to evaluate the symptomatic benefits of short-term nicotine administration, as detailed in section VII.A, nicotine's potential to protect against neuronal damage in Parkinson's disease is not known. A recent Michael J. Fox Foundation-funded international clinical trial vetting the potential of a nicotine skin patch to modify the progression of Parkinson's disease should provide some answers to this important question.

VII. Nicotine and Symptomatic Improvement

A. Patients with Parkinson's Disease

A number of reports and small clinical trials over the last 2 decades show that nicotine treatment reduces motor symptoms in patients with Parkinson's disease (Ishikawa and Miyatake, 1993; Fagerström et al., 1994; Kelton et al., 2000; Mitsuoka et al., 2002; Hanagasi et al., 2007). However, these are counterbalanced by an approximately equal number of studies that demonstrate no improvement in clinical features with either nicotine or a nicotinic agonist (Clemens et al., 1995; Vieregge et al., 2001; Lemay et al., 2004; Parkinson Study Group, 2006) or even a slight worsening of symptoms (Ebersbach et al., 1999). A comparison of the different studies was done with the intent of identifying factors that may account for the positive outcomes (Table 3). Careful analyses suggested that improvement did not correlate with nicotine formulation, dosage, or treatment paradigm. Instead, the most obvious success indicator seemed to relate to study design. Improvement was observed in studies with only a small number of pa-

TABLE 3
Clinical improvement of Parkinsonism with nicotine treatment correlates best with study design

Parkinsonism was assessed by measurement of a variety of different outcomes, including testing of fine motor skills, tremor, rigidity, bradykinesia, posture, hand and finger dexterity, or by using the following rating scales: the Unified Parkinson's Disease Rating Scale, Hoehn and Yahr, Columbia University, and Schwab-England.

Study Design	Improve Parkinsonism	Nicotine Formulation	No. of Patients	Treatment Regimen			References
				Duration of Dose Titration	Duration of Maintenance Dose	Maintenance Dose/Day	
Open-label	Yes	Smoking/ nicotine gum	6		Chronic smoker		Ishikawa and Miyatake, 1993 Kelton et al., 2000
	Yes	Intravenous nicotine and patch	15	2 weeks	1-2 weeks	14 mg	
	Yes	Nicotine gum	8	N.D.	1 day	N.A.	Mitsuoka et al., 2002 Hanagasi et al., 2007 Villafane et al., 2007
	Yes	Smoking	1		Chronic smoker		
	Yes	Nicotine patch	6	14 weeks	4 weeks	Up to 105 mg	
Double-blinded	No	Nicotine patch	22	22 day	3 day	21 mg	Lemay et al., 2004
	No	Nicotine gum	48	N.D.	1 day	3 × 2 mg	Clemens et al., 1995
	No	Nicotine patch	16	N.D.	12 h	7 mg	Ebersbach et al., 1999
	No	Nicotine patch	32	1 week	2 weeks	14 mg	Vieregge et al., 2001
	No	SIB-1508Y	77	2 weeks	2 weeks	10 mg	Parkinson Study Group, 2006
	Yes	Nicotine gum and patch	2		≥7 months	15 mg patch + 4 × 4 mg gum	Fagerström et al., 1994

N.D., not determined; N.A., not available.

tients (1–15 patients), with no effect of nicotine on Parkinson's disease symptoms in the larger study groups (16–77 patients). In addition, improvement seems to correlate with an open label but not placebo-controlled protocol. Although initially unexpected, this latter outcome is consistent with work that demonstrates significant improvements in Parkinson disease symptoms with placebo treatments, with a 9 to 59% placebo effect across different trials (de la Fuente-Fernández and Stoessl, 2002; Goetz et al., 2008). A large, recent analysis involving 858 patients with Parkinson's disease on placebo demonstrated an overall placebo response rate of 16%, with a range of 0 to 55% (Goetz et al., 2008). These findings highlight the importance of including a placebo group in drug and other experimental trials.

B. Parkinsonian Animal Models

The clinical data demonstrating inconsistent effects of nicotine on Parkinson's disease motor symptoms are in agreement with results in parkinsonian animal models (Table 4). Work with unilateral 6-hydroxydopamine-lesioned rats or mice show that neither short- nor long-term nicotine treatment modified parkinsonism either with or without L-DOPA treatment (Bordia et al., 2008, 2010; Huang et al., 2011). In these experiments, the effect of nicotine on motor function was tested only after toxin-induced nigrostriatal damage was complete. In another study, nicotine pretreatment did improve dopamine agonist-induced turning behavior after lesioning; however, data interpretation is complicated by the fact that nicotine pretreatment may have protected against nigrostriatal damage (Meshul et al., 2002). Nicotine or nicotinic agonists have also been tested on motor deficits in nonhuman primate models with variable effects. Long-term nicotine administration did not alter parkinsonism in MPTP-lesioned monkeys (Quik et al., 2006b). However, in other monkey studies, short-term nicotine injection did improve some motor deficits (Domino et al., 1999), and nicotinic agonist treatment acted synergistically with L-DOPA to ameliorate parkinsonism (Schneider et al., 1998).

In summary, the data from patients with Parkinson's disease and parkinsonian animal models have yielded con-

flicting outcomes concerning the efficacy of nicotine in improving motor problems linked to nigrostriatal damage. The discrepant nature of the results suggest that nicotine does not improve Parkinson's disease motor symptoms or that nicotine is of benefit only under specific conditions that remain to be defined.

VIII. Nicotine and L-DOPA-Induced Dyskinesias

A. L-DOPA Treatment for Parkinson's Disease Induces Dyskinesias

As stated earlier, the most prominent problem in Parkinson's disease is the motor deficits, which can effectively be treated with dopamine replacement therapies. The "gold standard" treatment is with the dopamine precursor L-DOPA, which greatly improves quality of life particularly in the early stages of the disease. However, long-term L-DOPA use results in the development of side effects such as dyskinesias (Fahn, 2008; Lang, 2009; Poewe, 2009; Schapira et al., 2009; Pezzoli and Zini, 2010). These abnormal involuntary movements of the head, trunk, and/or extremities interfere with functions of daily living and may become very debilitating. They are relatively common with approximately a 30% incidence after 2 years of L-DOPA treatment, 40% by 5 years, and 90% by 10 years (Ahlskog and Muenter, 2001). A variety of strategies have been proposed to minimize their onset, including a delay in the initiation of L-DOPA therapy, reduction in L-DOPA dose and/or treatment with dopamine agonists (Fahn, 2008; Lang, 2009; Poewe, 2009; Schapira et al., 2009; Pezzoli and Zini, 2010). However, there are drawbacks to these approaches, because modifications in L-DOPA treatment generally compromise the control of Parkinson's disease symptoms, whereas dopamine agonists fail to be as effective as L-DOPA and are associated with their own set of side effects. Amantadine, a drug with multiple CNS actions, including inhibition of NMDA glutamate receptors, is currently the only drug that is used clinically for the treatment of L-DOPA-induced dyskinesias, although its efficacy is limited. Continuous delivery therapies are under consideration to minimize the fluctuations in dopamine levels that may contribute to the development of dyskinesias. These include subcutaneous or intravenous dopamine agonist therapies, transdermal patches with dopamine agonists, or intraduodenal L-DOPA infusion. Other pharmacological treatments include concurrent L-DOPA treatment with catechol-O-methyl transferase and monoamine oxidase inhibitors to prolong L-DOPA effectiveness (Jankovic and Stacy, 2007; Fahn, 2008; Lang, 2009; Poewe, 2009; Schapira et al., 2009; Obeso et al., 2010). A surgical strategy that holds great promise in certain subsets of patients is deep-brain stimulation; however, its invasive nature is a limiting factor (Fahn, 2008; Benabid et al., 2009). Overall, the control of L-DOPA-induced dyskinesias is fairly limited. There is therefore a great unmet need to identify novel therapies.

TABLE 4
Variable effects of nicotine on parkinsonism in animal models with nigrostriatal damage

Animal Model & Change in Parkinsonism	Drug	Study Duration	Reference
6-OHDA-lesioned rat			
None	Nicotine	Weeks	Bordia et al., 2008
Improvement	Nicotine	Acute dose	Meshul et al., 2002
6-OHDA-lesioned mouse			
None	Nicotine	Weeks	Huang et al., 2011
MPTP-lesioned monkey			
None	Nicotine	Weeks	Quik et al., 2006b
Improvement	Nicotine	Acute dose	Domino et al., 1999
Synergistic with L-DOPA	SIB-1508Y	Acute dose	Schneider et al., 1998

6-OHDA, 6-hydroxydopamine.

Knowledge of the cellular and molecular mechanisms responsible for the occurrence of L-DOPA-induced dyskinesias would greatly facilitate in the development of targeted treatments. Indeed, this is an area under investigation in numerous laboratories. L-DOPA seems to mediate its initial effects via a dopamine receptor D1-mediated interaction. A current hypothesis is that dyskinesias arise because of an inability to turn down supersensitive signaling responses downstream of dopamine D1 receptors that arose in response to nigrostriatal dopaminergic denervation. DARPP-32 is one of the targets modified in L-DOPA-induced dyskinesia in rodents, with increased phosphorylation of its activating Thr34 site and several downstream targets including extracellular signal-regulated kinase and cFos (Fig. 5; see section III.C) (Picconi et al., 2003; Santini et al., 2007). The sustained activation of intracellular signaling pathways induced by multiple L-DOPA dosing leads to aberrant CNS activity that is behaviorally expressed as abnormal involuntary movements (Cenci and Konradi, 2010). This is compatible with the dependence of the molecular changes in L-DOPA-induced dyskinesia on supersensitive D1 receptor activation. This model implicates predominantly the "direct" output pathway that disinhibits the thalamus to promote motor commands (Fig. 3).

In addition to the dopaminergic system, evidence also suggests a prominent involvement of the serotonergic system in the occurrence of L-DOPA-induced dyskinesias. This idea is based on work showing that L-DOPA-induced dyskinesias are reduced with serotonergic denervation of the raphe nuclei or by treatment with selective serotonergic antagonists (Carta et al., 2007, 2008b; Eskow et al., 2007, 2009; Muñoz et al., 2008). Serotonergic inputs from the raphe to SN, and to a lesser extent to the striatum, the globus pallidus and the subthalamus are most likely involved (Di Matteo et al., 2008). Indeed, increased sprouting of serotonin terminals in the striatum is associated with the presence of L-DOPA-induced dyskinesias (Rylander et al., 2010). These data suggest that treatments that diminish serotonergic tone may be of clinical benefit in the management of L-DOPA-induced dyskinesias in patients with Parkinson's disease. The possibility of nicotinic modulation of this serotonergic system remains to be investigated.

In fact, research is in progress in numerous laboratories to investigate the antidyskinetic potential of drugs directed to various molecular targets linked to the nigrostriatal system, including the glutamatergic, GABAergic, adenosine, cannabinoid, noradrenergic, and other systems (Carta et al., 2008a; Fox et al., 2008; Jenner, 2008a,b; Cao et al., 2010; Lebel et al., 2010; Morin et al., 2010). Work is also being done to investigate the antidyskinetic potential of nicotine and nAChR-directed ligands in view of the extensive nicotinic cholinergic innervation in the nigrostriatal system (Quik et al., 2009).

B. Nicotine Decreases L-DOPA-Induced Dyskinesias in Parkinsonian Animal Models

Studies in several parkinsonian animal models show that nicotine treatment results in significant declines in L-DOPA-induced dyskinesias (Quik et al., 2007a; Bordia et al., 2008, 2010; Huang et al., 2011). Long-term nicotine dosing via several different modes of administration, including drinking water, minipump, and injection, decreased L-DOPA-induced dyskinetic movements in unilateral 6-hydroxydopamine-lesioned mice and rats and also in MPTP-lesioned monkeys, a model that bears many resemblances to Parkinson's disease. Tolerance did not seem to develop, with the effect of nicotine persisting with prolonged treatment time (months) (Quik et al., 2007a; Bordia et al., 2008, 2010; Huang et al., 2011).

An important question that arises is the subtype and location of nAChRs that underlie nicotine's ability to reduce L-DOPA-induced dyskinesias, because this may allow for the development of selective pharmacotherapies with minimal side effects. As summarized in the schematic in Fig. 6, the most relevant current targets seem to be the $\alpha 6\beta 2^*$ and $\alpha 4\beta 2^*$ nAChRs. We therefore tested the effect of agonists directed to these subtypes for their antidyskinetic potential in unilateral 6-hydroxydopamine-lesioned rats (Huang et al., 2011). Varenicline, an agonist that interacts with multiple nAChR subtypes (Mihalak et al., 2006; Rollema et al., 2007b), reduced L-DOPA-induced dyskinetic-like movements by 50% in parkinsonian rats. A similar decline was obtained with 5-iodo-A-85380, a nAChR agonist that interacts preferentially with $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs (see section III.A.1). These latter two nAChR populations may thus be involved in the antidyskinetic effect. The effectiveness of these novel nAChR agonists, and also of nicotine, seemed dependent on the degree of nigrostriatal damage, with reductions in dyskinesias with moderate (~30%) but not near-complete (~99%) nigrostriatal lesions (Huang et al., 2011). None of the drugs reduced the anti-parkinsonian action of L-DOPA, suggesting that the mechanisms by which nicotine modulates L-DOPA-induced dyskinesias are distinct from those involved in motor functions linked to parkinsonism.

In summary, nicotine and CNS agonists targeting $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR subtypes reduced L-DOPA-induced dyskinesias in a rodent model, the magnitude of the decline depending on the integrity of the dopaminergic system. These data suggest that $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR agonists may be useful for the treatment of L-DOPA-induced dyskinesias in Parkinson's disease.

IX. Conclusion and Future Directions

There is a consensus among epidemiologists that smoking is associated with a reduced incidence of Parkinson's disease. These observations led to the hypothesis that the nicotine in tobacco may play a role in protection against nigrostriatal damage because nicotine is well known to interact with the dopaminergic system. This idea was sub-

stantiated by studies showing that nicotine treatment protected against neurotoxin-induced nigrostriatal damage in both rodent and nonhuman primate parkinsonian animal models. Converging evidence thus supports a clinical trial to determine whether nicotine reduces Parkinson's disease progression.

Studies have also been done to investigate short-term effects of nicotine on Parkinson's disease motor symptoms because nicotine is well known to modulate dopaminergic function in short-term studies. The results of this work are less clear with improvement in motor deficits in approximately half of the clinical trials and only in some studies involving parkinsonian animal models. These conflicting results may suggest that nicotine is ineffective or that it possibly attenuates symptoms only under specified experimental conditions that remain to be identified. Further work is necessary to resolve this controversy.

More recent developments indicate that nicotine administration may be useful as an adjunct therapy to minimize L-DOPA-induced dyskinesias, a troubling side effect of L-DOPA therapy. Evidence for this stems from animal studies showing that nicotine reduces L-DOPA-induced dyskinesias in several parkinsonian animal models including rats, mice, and monkeys. These experimental observations suggest that a clinical trial testing the effect of nicotine may be warranted. Several nicotine formulations are currently available as smoking cessation aids, including the nicotine patch, gum, lozenge, nasal spray, and nasal inhalant. One or more of these applications may have potential for reducing L-DOPA-induced dyskinesias in Parkinson's disease. The beneficial effects also seen with varenicline, a synthetic drug licensed for use in smoking cessation, against L-DOPA-induced dyskinesias in animal studies encourages the view that there is a therapeutic niche for new nicotinic ligands.

An outstanding gap at the present time is whether nicotine exerts its beneficial effects in Parkinson's disease by acting at select nAChR subtypes and whether the same subtypes mediate the neuroprotective and the antidyskinetic effects of nicotine. This knowledge is important as it may lead to directed therapies with optimal therapeutic benefits and minimal side effects. Initial studies suggest that drugs directed to $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR populations present on nigrostriatal dopamine terminal neurons, and notably on their afferents projecting to the striatum, may be most relevant both for symptomatic therapy and long-term protection against nigrostriatal damage. Further studies to identify the most relevant nAChR subtypes, and their signaling pathways may reveal novel drug targets to combat Parkinson's disease.

Another gap in current knowledge relates to the most effective nAChR drug formulation for Parkinson's disease management. nAChR agonists are well known to initiate a rapid receptor desensitization with a consequent functional blockade. The question that arises is whether nAChR agonists exert their beneficial effects via receptor activation, or by blocking receptor mediated activity, in

which case antagonists may be more useful from a clinical standpoint. Alternatively, partial nAChR agonists (such as varenicline) may be more effective therapeutically. Continued research is required to address these possibilities.

Although not a focus of the current review, nonmotor symptoms are increasingly recognized as a significant problem in patients with Parkinson's disease (Hawkes, 2008; O'Sullivan et al., 2008; Schapira, 2009; Obeso et al., 2010). These include olfactory dysfunction, sleep disorders, cognitive declines, depression, and pain. To our knowledge, studies have not yet been done to evaluate whether nicotine may relieve such symptoms in Parkinsonian animal models or in patients with Parkinson's disease. However, nAChR drugs have been reported to modulate neuronal circuits involved in some of these deficits to facilitate cognitive performance, reduce pain, and alleviate depression (Bacher et al., 2009; Buckingham et al., 2009; McIntosh et al., 2009; Poorthuis et al., 2009; Sarter et al., 2009; Changeux, 2010a; Mineur and Picciotto, 2010; Philip et al., 2010). Thus, the use of nAChR drugs may not only protect against nigrostriatal damage and reduce L-DOPA-induced dyskinesias but also prove beneficial against nonmotor symptoms associated with Parkinson's disease. Future work is necessary to address this gap.

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Wrote or contributed to the writing of the manuscript: Quik and Wonnacott.

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