Striatal Cholinergic interneurons in the dorsal and ventral striatum: anatomical and functional considerations in normal and diseased conditions

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Abstract

Striatal cholinergic interneurons (ChIs) are central for the processing and reinforcement of reward-related behaviors that are negatively affected in states of altered dopamine transmission, such as in Parkinson’s disease or drug addiction. Nevertheless, the development of therapeutic interventions directed at ChIs has been hampered by our limited knowledge of the diverse anatomical and functional characteristics of these neurons in the dorsal and ventral striatum, combined with the lack of pharmacological tools to modulate specific cholinergic receptor subtypes. This review highlights some of the key morphological, synaptic, and functional differences between ChIs of different striatal regions and across species. It also provides an overview of our current knowledge of the cellular localization and function of cholinergic receptor subtypes. The future use of high-resolution anatomical and functional tools to study the synaptic microcircuitry of brain networks, along with the development of specific cholinergic receptor drugs, should help further elucidate the role of striatal ChIs and permit efficient targeting of cholinergic systems in various brain disorders, including Parkinson’s disease and addiction.

Keywords

accumbens; caudate nucleus; putamen; cocaine; Parkinson’s disease; drug addiction

Introduction

The basal ganglia are a group of tightly interconnected subcortical nuclei that regulate various aspects of sensorimotor, cognitive, and limbic functions.\(^1\) Disorders involving these brain structures encompass many neurodegenerative and neuropsychiatric syndromes.\(^2-4\)

The striatum, which consists of the caudate nucleus, putamen, and nucleus accumbens, serves as the main entry point for highly topographic and functionally segregated cortical...
information to gain access to the basal ganglia circuitry.\textsuperscript{1, 5} Thus, the cerebral cortex imposes a functional compartmentalization of sensorimotor (postcommissural putamen), associative (caudate nucleus and precommissural putamen), and limbic (nucleus accumbens) processes upon the striatum, which are partly maintained throughout the basal ganglia–thalamocortical loops.\textsuperscript{1, 5} Complex intrastriatal microcircuits that involve two populations of GABAergic projection neurons (also called medium spiny neurons (MSNs)), various groups of GABAergic interneurons, and a single population of cholinergic interneurons integrate, process, and transmit this extrinsic information to other basal ganglia nuclei.\textsuperscript{6–8} Despite the low number of striatal interneurons,\textsuperscript{6, 9, 10} they prevail as essential nodes of normal basal ganglia function because of their neurochemical diversity, intricate connectivity with MSNs, and central role in modulating striatal afferents.\textsuperscript{6} This review will focus on the striatal cholinergic interneurons (ChIs), the largest cells in the striatum that are recognized for their key regulatory roles of striatal and basal ganglia function in normal and diseased states.\textsuperscript{11–19}

Together with ventral midbrain dopaminergic neurons and their widespread striatal innervation, ChIs mediate aversive, attentional, motivational, and reward-related behaviors, as well as synaptic plasticity, conditioned learning, and action selection in the striatum.\textsuperscript{12, 14, 20–23} A striatal dopamine/acetylcholine imbalance has been discussed in numerous reviews as a key neurochemical substrate for basal ganglia disorders, such as Parkinson’s disease (PD) and drug addiction/abuse.\textsuperscript{11, 12, 16, 17, 20} Although significant advances have been made toward delineating ChI function in such disorders, the development of potential cholinergic drug therapies has been hindered by incomplete and controversial knowledge about the network connectivity of ChIs, along with a limited number of pharmacological tools that regulate specific subtypes of cholinergic receptors. In addition, striatal ChIs are often mistakenly assumed to represent a homogeneous population of cells with minor morphological and functional differences between humans and other animal species, and across different striatal regions.

This review discusses evidence for interspecies and regional differences in the morphology, abundance, distribution, connectivity, and physiological activity of ChIs in the caudate nucleus, putamen, and nucleus accumbens, along with some attributes of these neurons that are common to all mammals and striatal regions. The potential impact of striatal cholinergic dysfunction in diseased states, particularly PD and cocaine addiction/abuse, is then considered, and possible therapeutic targets for these disorders are examined. We conclude with a brief discussion about the limitations of the current cholinergic and dopaminergic therapies, and highlight the potential clinical relevance of new drugs aimed at specific cholinergic receptor subtypes, or receptors expressed on ChIs themselves, as potential therapeutics for PD and drug addiction.

**Striatum: functional compartmentalization and afferent connections**

**Striatal compartmentalization**

The striatum is the main entryway through which extrinsic afferents can influence functionally diverse basal ganglia circuits to generate context-dependent, goal-directed, and habitual behaviors.\textsuperscript{24–26} It is commonly divided into the dorsal and ventral striatum on the basis of gross anatomical localization and divergent connectivity. In primates, the dorsal
The striatum consists of the caudate nucleus and putamen, divided from each other by the internal capsule, whereas in rodents, it is a single mass of gray matter often referred to as the caudate–putamen complex. The ventral striatum consists of the nucleus accumbens and the striatal portion of the olfactory tubercle, along with the ventromedial extension of the caudate nucleus and putamen. The nucleus accumbens comprises a core and a shell subregion, two anatomically and functionally defined areas that are well-characterized in rodents, but less so in primates. In general, the rostral-most, medial, lateral, and ventral parts of the accumbens are referred to as the shell, while its dorsal and central portions constitute the core.

Another level of striatal compartmentalization is the patch (or striosomes)/matrix system, which is largely based on the heterogeneous distribution of neurochemical markers and differential afferent or efferent connections. The two compartments have been identified within the dorsal and ventral striatum of primates and nonprimates, but they are particularly evident in the caudate nucleus, anterior putamen, and core of the accumbens. Although the functional significance of this patch/matrix dichotomy remains poorly understood, evidence that these two compartments are differentially affected in some basal ganglia disorders is of significant interest.

Afferent connections to the striatum

**Corticostriatal system**—Extrinsic topographically and functionally organized projections from the cerebral cortex, thalamus, and ventral midbrain constitute the bulk of striatal afferents. Cortical glutamatergic projections terminate throughout the whole striatum in a functionally topographic fashion. In primates, the postcommissural putamen (or dorsolateral striatum in rodents) receives its main cortical inputs from sensorimotor cortices, while the precommissural putamen and the caudate nucleus (or dorsomedial striatum in rodents) are the main targets of cognitive afferents from associative prefrontal, temporal, and parietal cortical regions. These sensorimotor and cognitive inputs terminate predominantly within the matrix sector of the caudate nucleus and putamen. In contrast, the striosomes of the dorsal striatum receive their main cortical innervation from limbic cortices (such as the orbitofrontal, anterior cingulate, and insular cortices) and the amygdala, which, together with the hippocampus, are the main sources of cortical and subcortical inputs to the ventral striatum. In rodents, clear evidence exists for a differential origin of limbic-related cortical afferents to the core and shell of the accumbens. Although such a dichotomy also exists in primates, the less distinct borders between the accumbens core and shell in monkeys and humans makes the distinction between these two regions more difficult to establish.

**Thalamostriatal system**—The thalamus is the other major source of glutamatergic inputs to the striatum. Although the rostral intralaminar (mainly the centrolateral nucleus), ventral motor (ventral anterior/ventral lateral nuclei), associative (mediodorsal nucleus), and midline thalamic nuclei contribute to the thalamostriatal system, the main origin of these projections are the caudal intralaminar nuclei, namely the centre median (CM) and parafascicular (Pf) nuclei, that project primarily to the sensorimotor (CM inputs) and associative/limbic (Pf inputs) dorsal striatal territories in primates. In non-primates, the...
intralaminar (and most other thalamic nuclei) preferentially target the matrix region of the caudate–putamen complex, while the patch/striosomes receive their main thalamic innervation from the paraventricular (PV) nucleus.\textsuperscript{53, 54} The PV, parataenial, Pf, and mediadorsal thalamic nuclei constitute the main sources of thalamic inputs to the ventral striatum across species.\textsuperscript{50, 55–57} Within subregions of the monkey accumbens, the core (i.e., the dorsal centrolateral region) receives a dense innervation from the Pf with limited inputs from the midline thalamic nuclei, whereas the shell (i.e., medial region) is the recipient of afferents from both of these thalamic nuclear groups.\textsuperscript{56} Like the corticostriatal system, the thalamostratial projections to the caudate nucleus, putamen, and nucleus accumbens are functionally topographic,\textsuperscript{52, 58} and in the case of CM/Pf, part of functionally-segregated basal ganglia–thalamostratial loops that process sensorimotor, associative, and limbic information.\textsuperscript{52, 58, 59} However, a certain level of integration and convergence exists within and between the various structures involved in these complex circuits, particularly at the level of associative and limbic loops.\textsuperscript{5, 60, 61}

**Mesostriatal dopaminergic systems**—In addition to glutamatergic inputs, the mammalian striatum is the target of strong dopaminergic afferents from the substantia nigra pars compacta (SNc), the ventral tegmental area (VTA), and the retrorubral area (RRA).\textsuperscript{44, 62–66} In general, the VTA and medial SNc mainly project to the limbic striatum, the lateral SNc innervates the associative and sensorimotor striatum, while the mid-central SNc and RRA send afferents to all three functional regions of the dorsal and ventral striatum in all species.\textsuperscript{64, 66, 67} In rats, the medial and lateral regions of the dorsal SNc innervate the matrix compartment of the associative and sensorimotor striatum, respectively, whereas the patches receive their main dopaminergic innervation from the medial and lateral aspects of the ventral SNc.\textsuperscript{66, 68, 69} Despite this general topographical arrangement, it is noteworthy that single dopaminergic axons often innervate neurons in both striatal subcompartments.\textsuperscript{70} It is currently unknown if similar relationships exist between the patch–matrix compartments and the mesostriatal dopaminergic systems in primates.\textsuperscript{71–75}

**Cholinergic systems**—Although the bulk of acetylcholine in the striatum is released by intrinsic striatal ChIs, the brainstem pedunculopontine nucleus (PPN) and the laterodorsal tegmental (LDT) region also provide significant cholinergic inputs to the mammalian striatum.\textsuperscript{76} Early evidence for this projection from retrograde-tracing studies in various species,\textsuperscript{62, 77–80} has recently been confirmed and expanded in choline acetyltransferase (ChAT)-Cre rats using AAV2-YFP as an anterograde tracer.\textsuperscript{76} Results of this study showed a significant, topographically organized projection from various subdivisions of the PPN/LDT complex to the sensorimotor and limbic regions of the rat striatum. This striatal projection originates from PPN/LDT neurons that also provide cholinergic innervation of the thalamus and ventral midbrain, suggesting that this ascending cholinergic system can modulate striatal activity through both direct striatal projections or indirectly via modulation of the thalamostratial and mesostriatal systems. At the ultrastructural level, the majority of cholinergic terminals from PPN/LDT formed asymmetric synapses with spines and dendritic shafts of striatal projection neurons,\textsuperscript{76} a pattern of connectivity different from the symmetric synapses formed by terminals of intrastratial ChIs.\textsuperscript{81–85} Future studies are needed to assess the functional significance of this extrinsic cholinergic innervation to the striatum.
Other striatal afferents—Additional inputs to the dorsal and ventral striatum originate from GABAergic neurons in the globus pallidus (GP), \(^{86-88}\) and the subcommissural ventral pallidum (VP), \(^{89-91}\) respectively, along with those from serotonergic neurons in the dorsal raphe, \(^{48, 62, 92}\) noradrenergic neurons in the locus coeruleus (mainly to the ventral striatum), \(^{62, 93-95}\) histaminergic neurons in the hypothalamus, \(^{96, 97}\) and glutamatergic neurons in the subthalamic nucleus. \(^{62, 98}\)

**Striatum: cellular organization and efferent connections**

**MSNs of the dorsal striatum: direct and indirect pathways**

In rodents, 90–95% of dorsal and ventral striatal neurons are GABAergic projection MSNs, while the remaining striatal neuronal population consists of interneurons. \(^{9, 99, 100}\) A similar cellular organization exists within the primate dorsal striatum, although the proportion of interneurons is larger in primates than non-primates. \(^{6, 8-10, 101}\) Two populations of dorsal striatal MSNs have been categorized on the basis of their projection sites, neuropeptide expression, and dopamine receptor content. The MSNs that project directly to the basal ganglia output nuclei (i.e., the internal segment of the GP (GPi) and the substantia nigra pars reticulata, (SNr)), referred to as direct-pathway neurons, predominantly express substance P (SP), dynorphin, and D1 dopamine receptors. On the other hand, striatal MSNs that project to the external segment of the GP (GPe), referred to as indirect-pathway neurons, contain enkephalin (Enk) and preferentially express the D2 dopamine receptors. \(^{34, 101, 102}\) Although this segregation of striatal output neurons is the basis for functional models of information flow through the basal ganglia circuits, it is oversimplified because a small, but significant, subset of MSNs co-express D1 and D2 dopamine receptors (and possibly D3 and D4 receptors), and a number of striatal MSNs send axonal projections to both the GPe and GPi (or SNr) in rats and monkeys. \(^{20, 103-105}\)

**MSNs of the accumbens core and shell: overlapping efferent connectivity**

Although the dorsal and ventral striatum are similar in many respects, the cellular composition of the ventral striatum tends to be more heterogeneous. For example, distinct clusters of neurons referred to as interfaced islands, consisting of the insula major of Calleja and the islands of Calleja, reside in the accumbens and olfactory tubercle, respectively, but not in the dorsal striatum. \(^{106, 107}\) In addition, the classification of accumbal MSNs into only two categories (i.e., direct and indirect) is not straightforward because of their overlapping efferent projection patterns and the complexity of the shell/core compartmentalization. \(^{108-110}\)

Similarly to the efferents from the dorsal striatum, ventral striatal projections primarily target the pallidum and the ventral mesencephalon. \(^{44, 111}\) but projections from the accumbens shell also innervate non-basal ganglia structures, such as the lateral preoptic and hypothalamic areas, the mediodorsal thalamic nucleus, the pedunculopontine nucleus (PPN), the medial central gray, the bed nucleus of the stria terminalis, and the nucleus basalis. \(^{44, 111}\) In contrast to the dominant accumbal projection to the ventral pallidum in rodents, accumbal efferents in primates provide an equally large innervation of both the dorsal and ventral pallidum. \(^{111-113}\) In particular, accumbal MSNs topographically project to the rostral pole of
the GPe, the rostromedial portion of the GPi, and the subcommissural VP, implying that the rostral and medial GP has a distinct association with the ventral striatum in monkeys.44,111 The accumbal efferents to the nigra (i.e. both the SNC and SNR) and VTA follow a loose topographical organization that permits the integration of information from different accumbal regions across the entire ventral mesencephalon in primates.44, 67, 111 Overall, the accumbal projection to the SNC is more massive than to the VTA, most particularly in primates, suggesting that nigrostriatal neurons that innervate sensorimotor and associative striatal regions are tightly regulated by limbic-related signals from the ventral striatum.44, 67, 111 Conversely, the accumbal projections in rats follow a strict topography, consisting of core and shell MSNs that project either to the SNr or to the SNC, VTA, and retrorubral neurons, respectively.114

**Striatal interneurons**

GABAergic neurons that express parvalbumin, somatostatin/neuropeptide Y/nitric oxide synthase, calretinin or tyrosine hydroxylase (TH), and non-GABAergic cholinergic cells, represent the main populations of striatal interneurons in the dorsal and ventral striatum of primates and non-primates,10, 115–120 Overall, interneurons account for about 5–10% of the total neuronal population in rodents, but this proportion is significantly higher in primates.9 In the rat striatum, ChIs account for approximately 1% of the total neuronal population (absolute number is unknown),85 but the proportion of ChIs in the primate dorsal striatum has not been thoroughly quantified.121, 122 Similarly, the proportion of striatal neurons accounted for by ChIs in the rodent and primate nucleus accumbens remains to be determined using unbiased stereological methods.

**Heterogeneous morphology of striatal cholinergic interneurons**

**ChIs in the dorsal striatum**

**Morphological, ultrastructural, and cytological features of ChIs**—Although both ChAT immunoreactivity and acetylcholinesterase (AchE) staining have been used as markers of putative ChIs in primates and rodents, some interspecies differences in AchE expression are worth noting. In primates, there is a tight correlation between the two markers, with both being exclusively expressed in a population of large neurons with similar morphology and frequency.123 However, three populations of AchE-positive neurons have been identified in the rodent dorsal striatum, only one of those being immunoreactive for ChAT.124–128

The morphology and size of ChIs in the dorsal striatum differs between primates and non-primates. Immunoreactive cell bodies for ChAT- or AchE-containing neurons in the primate dorsal striatum have an average diameter of 35 μm and display various shapes (Figs. 1A–C and 2F–H),7, 82, 123, 129 whereas their cell bodies are smaller (around 25 μm) and mostly oval in rodents (Fig. 2A–E and Table 1).85, 120, 124, 126 In addition to these somatic differences, ChIs in the rat caudate–putamen complex have more somatic and dendritic spines, less primary dendrites, and a sparser ramification of their distal dendritic trees (Fig. 2A–E)21, 85, 120, 124–126, 130 compared with monkeys (Figs. 1A–C and 2G) and humans (Fig. 2F).7, 82, 123, 127, 129, 131–134 Through correlations between the morphology of Golgi-filled...
neurons and ChAT- (or AchE)-positive cells in the primate dorsal striatum, ChIs have been described as having a “spidery” appearance because of their large cell bodies from which emerge thick (up to 10 μm) primary dendrites that give rise to profuse “spider-like” dendritic trees and widespread intrastriatal axonal arborizations (Figs. 1A–C and 2F–H).7, 82, 123, 127, 129, 131–134 On the other hand, a similar type of analysis performed in the rodent striatum demonstrated that “spidery” cells correspond to only a small proportion of putative ChIs (Fig. 2C–E).124–128 In fact, even these apparent spidery neurons in the rodent striatum (Fig. 2C–E) display major morphological differences from ChIs in primates (Fig. 2F–H). For example, the spidery neurons in rats have a smaller number of dendritic tips (21–28 in rats versus over 100 in monkeys) and a significantly shorter total dendritic length (1,400–2,500 μm versus 23,400 μm) than in monkeys,127, 129 suggesting that the extent of synaptic innervation of, and extrinsic information processing by, ChIs is more complex in primates than in rodents.

Evidence from our laboratory and others shows differences in the shape and size of cell bodies across functional striatal regions in primates. For instance, ChAT-labeled neurons in the ventromedial caudate nucleus and the entire extent of the medial portion of the putamen have round or elongated cell bodies, as well as sparsely-branched dendritic trees (Fig. 1C), while cholinergic neurons in the remainder of the caudate nucleus (i.e., dorsomedial, mid, and lateral regions) and the whole lateral putamen display the classical spidery appearance (Fig. 1A and B). In rats, one research group has recently shown that ChAT-immunoreactive neurons in the dorsolateral and ventromedial striatum exhibit morphologically similar characteristics,120 which resemble those of primate ChIs in the ventromedial caudate nucleus and medial putamen (Fig. 1C). In humans, the volume of ChI cell bodies differs between regions of the dorsal striatum.7 ChIs with the largest soma volumes reside in the striatal regions that have the lowest density of these neurons—the caudate gyrus and the precommissural caudate nucleus and putamen—suggesting that the increased size of ChIs perikarya in these areas may compensate for their lower neuronal densities.7

There is a general assumption that ChAT-positive neurons belong to a single cell population in the dorsal striatum of primates and non-primates because most ChIs display a non-GABAergic phenotype135, 136 and common subcellular features, such as deeply indented nuclei and a richly embedded cytoplasm that contains rough endoplasmic reticulum, subsurface cisternae, lipofuscin granules, and large dense bodies.82, 85, 124–126, 131, 137, 138 However, the morphological and neurochemical features discussed above suggest a much more diverse group of neurons with higher capabilities of information integration in primates than in non-primates. Another striking difference between ChIs in rodents and primates relates to their differential expression of the calcium binding protein, calretinin. A subset of striatal ChIs co-express calretinin in primates, but not in rats and mice.93, 122, 139 Although the functional significance of this colocalization remains to be established, it is noteworthy that the number of calretinin/ChAT–positive neurons is reduced in the striatum of Huntington’s disease patients.139 Thus, the significant interspecies and intrastriatal morphological differences of these neurons (Fig. 1) should be taken into account when anatomical and functional data gathered from rodent ChIs are translated to the primate striatum.
Relative density of ChIs in the dorsal striatum—The most comprehensive stereological assessment of ChAT-immunoreactive neuron densities across all striatal territories has been performed by Bernacer et al. in the human caudate and putamen.⁷ According to this study, significant differences in the prevalence and pattern of distribution of ChI cell bodies are found between cortically defined functional territories of the dorsal striatum.⁷ The associative striatum (i.e., dorsomedial sector of the caudate head, body, and gyrus, and precommissural putamen) harbors a larger density of ChIs than the sensorimotor (i.e., dorsolateral caudate head, dorsal precommissural putamen, and postcommissural putamen excluding its ventral portion) and limbic (i.e., ventral caudate head and putamen) striatal regions.⁷ The density of ChIs was also shown to follow a positive rostrocaudal gradient in all functional areas of the human striatum in that the density of ChIs in precommissural striatal regions is lower than in postcommissural sectors of the striatum.⁷ Although similar rigorous quantitative analyses of ChI prevalence in the rodent striatum have not been performed, some authors have suggested that ChAT-labeled cell bodies are more densely distributed in the rostral than in the caudal regions of the caudate–putamen complex in rats.⁸⁵ If this is the case, it would represent another striking difference in the anatomical organization of striatal ChIs between rodents and primates (Fig. 3).

A common feature of ChIs in rats, monkeys, and humans is their predominant localization within the striatal matrix compartment, often at the striosomal borders, where their axons and dendrites cross over the patch–matrix boundaries, providing them a unique position to facilitate cross-communication between MSNs of the different striatal subcompartments and functional territories.⁷, ³⁸, ¹⁴⁰–¹⁴² Furthermore, in primates, ChAT-labeled neurons often reside in the striatal tissue bridges that cross the internal capsule between the caudate nucleus and putamen.

Dorsal striatal cholinergic neuropil—In addition to ChAT-immunoreactive cell bodies and dendrites, a dense meshwork of fine ChAT-labeled processes that most likely represent thin axons, small distal dendritic processes, and axon terminals of ChIs and brainstem afferents occupies the entire striatum (Figs. 1 and 3).¹²³, ¹³⁴, ¹⁴² Three main features characterize this rich cholinergic neuropil in the monkey and human striatum. First, it displays a patchy appearance made up of pockets of light immunostaining embedded within a field of denser immunoreactivity, reminiscent of the striatal patch/striosome compartment (Fig. 3).⁷, ¹²³, ¹⁴⁰, ¹⁴³ Second, it follows a positive rostrocaudal gradient in labeling intensity (Fig. 3A–C).¹²³ Third, areas of denser ChAT immunostaining lay within the medial parts of the precommissural and commissural caudate and putamen (Fig. 3A and B) and the lateral borders of the postcommissural putamen (Fig. 3C).⁷, ¹⁴² In contrast, the rodent ChAT-immunolabeled neuropil is rather homogeneous,⁸⁵, ¹²⁴ except for an increased labeling intensity (of both somata and neuropil) in the lateral border and most rostral regions of the striatum.⁸⁵

ChIs in the nucleus accumbens

Morphological, ultrastructural, and cytological features of accumbal ChIs—ChAT-labeled somata in the nucleus accumbens are either elongated or round in both primates and rodents with an average size smaller than those in the dorsal striatum (Table 1;
Similar to the dendritic trees of dorsal striatal ChIs (Fig. 1A–C), accumbal ChAT-labeled dendrites extend over long distances within the neuropil but are thinner and less profusely arborized than those in the dorsal striatum in both rodents and primates (Fig. 1D–G). At the ultrastructural level, ChIs in the ventral and dorsal striatum of rats display similar features, but such comparisons remain to be made in primates.

ChIs neuronal density and cholinergic neuropil labeling in the accumbens—ChAT immunolabeling displays greater heterogeneity in the nucleus accumbens than in the dorsal striatum across species (Figs. 3 and 4). In particular, rodent studies have shown a larger density of ChAT-labeled neurons and a stronger level of ChAT immunoreactivity in the shell neuropil (mainly the medial part) than in the core of the nucleus accumbens. Although differences have been suggested in the number of ChAT-labeled neurons between the shell and core of the accumbens in primates, these data must be confirmed using unbiased stereological quantification and a clearer delineation of the two accumbal regions in primates.

The pattern of ChAT-labeled neuropil described in the rodent accumbens is reminiscent of that observed in primates (Figs. 1E and 4). Furthermore, the insula major of Calleja and the islands of Calleja or the so-called “interface islands” display the densest ChAT neuropil labeling in the ventral striatum of both primates (Figs. 1G and 4B, C, E, F) and non-primates. More specifically, ChAT-immunoreactive cell bodies reside at either the border or in the center of these strongly immunoreactive areas that become more complex in primates (Fig. 1G).

Physiological activity of striatal cholinergic interneurons

On the basis of common morphological, regional, and functional similarities, ChIs correspond to electrophysiologically characterized, tonically active neurons (TANs) in the dorsal striatum, although direct correspondence between these cell types remains to be fully established in primates. Two recent studies that combined juxtacellular labeling and in vivo extracellular recordings in anesthetized rats revealed that striatal neurons with the electrophysiological features of TANs displayed immunoreactivity for ChAT and the characteristic large-sized soma of ChIs. In agreement with anatomical evidence for the existence of two morphologically distinct populations of ChIs (i.e., non-spidery and spidery) in the rodent dorsal striatum, TANs with long, sparsely-branched dendritic trees (Fig. 2A–C) or thick, moderately-branched dendrites that tapered into finer processes have been described (Fig. 2D and E).

Besides their morphological characteristics, the firing properties of striatal TANs distinguish them from other neurons in the dorsal striatum. Striatal TANs in rodents and monkeys exhibit a large depolarized membrane potential (approximately −60 mV), tonic spike discharge around 2–10 spikes/s, broad spike waveforms, and diverse spiking patterns (i.e., regular, irregular, and bursting). In rodents, in vivo recording of TAN responses to current pulse injections or afferent stimulation have revealed that TANs display a strong spike-frequency adaptation and low-frequency
oscillations (1–5 Hz) that are regulated by intrinsic mechanisms\textsuperscript{148, 150, 157, 158, 165–170}. Through ion channels located along their entire somatodendritic domain\textsuperscript{171}, a spike-induced, calcium-dependent afterhyperpolarization deters TAN rapid spiking, while a prominent I\textsubscript{h} current–mediated sag conductance prevents sustained hyperpolarization of TANs\textsuperscript{148, 150, 157, 165, 167, 169, 170}. Indeed, the average firing rate of TANs is almost impossible to change using constant current injections or an artificial synaptic barrage\textsuperscript{168}. TANs fire spontaneously and maintain their diverse spiking patterns in the absence of current injections or signals from external inputs\textsuperscript{148, 160, 165–167, 172, 173}. The firing pattern of individual TANs also alternates between spiking patterns shaped by temporally-defined (< 5Hz) oscillatory mechanisms, afterhyperpolarization currents (slow, medium, and fast), and the release of neurotransmitters and/or neuromodulators from their synaptic inputs\textsuperscript{164–166, 168, 169}. Collectively, these data imply that both intrinsic membrane properties and synaptic afferents regulate the pattern of TAN spike timing and output\textsuperscript{19, 168}.

While the mechanisms responsible for TAN spiking properties in the primate striatum remain to be fully established, in vivo extracellular recordings of striatal TANs in awake monkeys demonstrated that afferent activation induces changes in TAN firing activity similar to those observed during reward-related behavioral learning. For example, TANs in the dorsal striatum (mainly recorded from the precommissural level) respond to sensory salient events during behavioral conditioning\textsuperscript{149, 164, 174–180} and to cortical (mainly the supplementary motor area) or thalamic (CM/Pf) stimulation\textsuperscript{181, 182}, with a triphasic response that includes an early excitation, a pause in activity, and a rebound excitation. However, the most common response of TANs to these events comprises only a pause and rebound excitation in spike discharge\textsuperscript{149, 164, 183–187}, which is reduced or abolished by the pharmacological blockade of thalamic (i.e., CM/Pf) afferents\textsuperscript{179}. In light of these findings and others, it appears that the firing activity patterns of primate TANs associated with behavioral learning is under the regulation of strong afferent connections from the CM/Pf and midbrain dopaminergic neurons, as is the case in the rodent dorsal striatum\textsuperscript{19}.

Even though most TANs throughout the primate dorsal striatum have a similar response profile to behavioral conditioning, several key features of this response may be dependent on the location of TANs within a particular striatal territory. For instance, there is evidence that striatal TANs that respond to an individual stimulus scheme (i.e., to only one stimulus type or to multiple types of stimuli) during reward-associated learning are occasionally clustered together in specific functional regions of the striatum\textsuperscript{149, 164, 188}. For instance, TANs in the precommissural putamen display more synchronous firing activity during classical conditioning events than those in the caudate nucleus\textsuperscript{184} while TANs in the ventromedial striatum (i.e., precommissural striatum dorsolateral to the nucleus accumbens) display slower average discharge rates and, possibly, larger responses to behavioral learning compared to those in other striatal regions in monkeys\textsuperscript{183, 184}. On the other hand, synchronized TANs in the monkey postcommissural putamen encode motivational instructions for goal-directed action learning, but respond to behavioral cues in a similar fashion as TANs in the precommissural striatum\textsuperscript{189}. In contrast to this heterogeneity of primate TANs, the firing rates and responses of TANs to behavioral conditioning in rats are
similar between the dorsolateral and ventromedial striatal territories,\textsuperscript{190} in agreement with an analogous morphology of ChIs amid these regions in rodents.\textsuperscript{120}

**TANs in the nucleus accumbens**

Albeit with slightly different firing properties, TANs also exist in the rodent nucleus accumbens. Accumbal TANs are often overlooked though, due to recordings of accumbal neurons being grouped together on the basis of their responses to various tasks and pharmacological manipulations, instead of their firing rate and pattern properties.\textsuperscript{191–198} In the instances of TAN characterization, the reported baseline firing rates of TANs in the accumbens were quite variable (0.6–12 spikes/s with a tendency to be in the lower range).\textsuperscript{190, 199–206} In those studies that utilized \textit{in vitro} optogenetic\textsuperscript{201, 204} or juxtacellular single-cell filling\textsuperscript{203} approaches, recorded accumbal TANs corresponded to ChAT-immunoreactive neurons in ChAT-Cre or glutamic acid decarboxylase (GAD)-Cre mice, respectively. Collective data gathered from \textit{in vitro} and \textit{in vivo} electrophysiological studies in rodents have revealed that accumbal TANs exhibit variable spontaneous activity and firing patterns, \textit{Ih} currents, and prolonged refractory periods.\textsuperscript{190, 200–204, 206}

TANs in the ventral striatum play a role in the negative symptoms associated with depression, such as anhedonia and despair,\textsuperscript{207} suggesting their role in emotional control.\textsuperscript{205} In regard to their responses during behavioral conditioning and afferent stimulation, TANs simultaneously recorded with microarray electrodes in rat accumbal slices have non-synchronous firing activity, regular firing patterns, and display a decrease in firing activity in response to high-frequency intra-accumbal stimulation.\textsuperscript{206} During a simple instrumental task, several \textit{in vivo} extracellularly recorded TANs in the rodent accumbal core respond to reward-related cues and an expected reward with attenuated firing activity, whereas an unexpected reward increases their spike discharge.\textsuperscript{190} TANs in the accumbens shell change their \textit{in vitro} firing pattern to irregular and bursty following training for a contingent Pavlovian task in rodents.\textsuperscript{208, 209} Accumbal TANs display a pause in their activity followed by an excitation in response to \textit{in vitro} and \textit{in vivo} optogenetic activation of GABAergic VTA projections,\textsuperscript{203, 210} known to have a role in salience processing.\textsuperscript{211} However, the \textit{in vivo} recordings of TANs in the ventromedial striatum (includes the ventromedial caudate–putamen complex and nucleus accumbens) during the acquisition of reward-related learning have revealed that these neurons have unique bidirectional outcome responses (i.e., excitation upon the learning and relearning of positive reward-related tasks, but inhibition after reward omission).\textsuperscript{205} These data demonstrate that TANs in both striatal regions can modify intrastriatal circuits for the learning of new associations, even though their responses to reward and other motivational-related stimuli highly differ.\textsuperscript{205} The physiological firing properties of accumbal TANs in monkeys and their responses during the learning of stimulus-outcome associations remain to be characterized.

**Are striatal TANs exclusively ChIs?**

Recent data have shown that other striatal interneurons, such as those that express parvalbumin or nitric oxide, may also display spontaneous tonic activity in the dorsal striatum,\textsuperscript{212–214} raising caution when identifying ChIs solely on the basis of their tonic discharge patterns. These findings were obtained from ChAT-Cre transgenic mice with
GFP-labeled ChIs (or other striatal neurons) combined with optogenetic brain activation. Although these experimental tools are instrumental in relating the physiological properties of striatal neurons (including TANs) with their chemical phenotype and their respective role in basal ganglia function/dysfunction, the fact that the Cre recombinase technology is exclusively applied in rodents makes findings obtained from these animals difficult to confirm in primates. Thus, without a reliable translation of the rapidly growing genetic technology from mice to primates, our understanding of circuit- and cellular-specific processes of chemically characterized neurons in the human brain may rely entirely on data gathered from the mouse brain.

Synaptic regulation of striatal cholinergic interneurons

In addition to the numerous studies that have provided valuable insights into the underlying regulatory mechanisms of striatal ChIs in primates and non-primates, the following discussion includes findings from our laboratory and others showing that GABAergic inputs represent a substantial source of synaptic innervation to primate ChIs. We propose that this GABAergic innervation may play a critical role in mediating communication between groups of ChIs or between ChIs and other striatal neurons or their extrinsic striatal afferents.

General synaptic innervation of cholinergic interneurons

Qualitative electron microscopic observations of either Golgi-impregnated spidery neurons or ChAT-immunoreactive cells in the primate dorsal striatum have revealed that ChIs receive symmetric and asymmetric synaptic inputs from morphologically heterogeneous terminal boutons indicative of diverse sources (Fig. 6). In a recent quantitative study, we demonstrated that 60% of all terminals in contact with ChIs in the monkey postcommissural putamen are GABAergic, while about 20% are putatively glutamatergic (asymmetric synapses/GABA-negative), and the remaining 20% are of unknown chemical phenotype (symmetric synapses/GABA-negative) (Fig. 5). While GABA-positive and GABA-negative terminals form symmetric synapses with the entire somatodendritic domain of ChIs, putative glutamatergic terminals form asymmetric synapses mainly with the distal (or thinner) dendrites of these neurons. Because such detailed quantitative assessment of rodent ChIs innervation has not been achieved, it is difficult to make direct comparisons between our findings and those reported in rodents. It appears though that terminals forming symmetric or asymmetric synapses innervate striatal ChIs in rats, with a predominance of inhibitory synapses onto their proximal parts. However, in contrast to monkeys, the proximal dendrites and cell bodies of ChIs receive substantial excitatory inputs in rats. The proportion and location of putative inhibitory and excitatory synaptic inputs to ChIs in the ventral striatum remain to be determined.

Glutamatergic regulation of cholinergic interneurons

**Glutamatergic inputs to ChIs**—Although the cerebral cortex and thalamus are the two main sources of glutamatergic inputs to the striatum, thalamostratial projections from the caudal intralaminar nuclei (i.e., the CM/Pf) are the predominant origin of glutamatergic
terminals in contact with ChIs in the primate and non-primate dorsal striatum. Various studies that aimed at assessing the synaptic relationships of ChIs have demonstrated that corticostriatal afferents (labeled with anterograde tracers from prefrontal and parietal cortices or vesicular glutamate transporter 1 (vGluT1) antibodies) provide a sparse innervation of the distal dendrites of ChAT-labeled neurons in rodents and monkeys. These ultrastructural findings are difficult to reconcile with in vitro and in vivo electrophysiological and pharmacological studies showing that cortical stimulation elicits short-latency excitatory responses in TANs and induces extracellular acetylcholine release in the striatum. Thus, it is likely that the unique intrinsic membrane properties of ChI dendrites allow them to respond to these sparse distal cortical inputs.

Currently, no studies have examined the physiological effects of neocortical or thalamic inputs to accumbal ChIs. However, tracing studies in rats have shown that hippocampal inputs from the subiculum provide only sparse and distal synaptic innervation of accumbal ChIs, as was found for neocortical inputs in the dorsal striatum. In regard to their thalamic innervation, tracing studies from two groups have resulted in contrasting findings. On one hand, some researchers have shown that inputs from the PV nucleus terminate onto the dendrites of accumbal ChIs in the medial shell, while another research group has demonstrated that terminals from PV, midline, and rostral intralaminar nuclei do not form direct synaptic contacts with accumbal ChIs. An explanation for this discrepancy could be that the anterograde tracer injection aimed at the PV nucleus in the former study slightly contaminated more posterior thalamic nuclei, such as the Pf, known to largely innervate dorsal striatal ChIs.

Thus, a prominent source of glutamatergic innervation to ChIs in the dorsal striatum stems from the CM/Pf complex in both primates and non-primates (Fig. 6), but the details of this thalamic innervation in the nucleus accumbens remain to be further clarified. Nevertheless, the exact proportions of cortical, thalamic, hippocampal, and amygdalar inputs at the level of single ChIs await further quantitative ultrastructural analyses in both the dorsal and ventral striatum.

**Glutamatergic receptors on ChIs**—In both the ventral and dorsal striatum, ChIs express various subtypes of ionotropic and metabotropic glutamate receptor protein and mRNA, which is consistent with a large number of pharmacological studies showing that direct or indirect activation of glutamatergic systems results in changes in acetylcholine release and/or the depolarization of dorsal striatal ChIs. Prefrontal cortical inhibition and hippocampal activation induce N-methyl-D-aspartate (NMDA)-mediated release of acetylcholine in the accumbens, most likely through indirect routes that involve...
glutamatergic, GABAergic, and dopaminergic neurotransmitter systems.\textsuperscript{240, 255–259} Other pharmacological studies suggested that thalamic inputs from Pf regulate ChIs activity predominantly through NMDA receptor activation, while the effects of cortical afferents are mainly mediated through AMPA receptor activation.\textsuperscript{237, 238, 251, 260} Because of the limited information on the synaptic relationships between subtypes of glutamate receptors and their presynaptic afferent terminals from the cerebral cortex or thalamus, the underlying substrate for these specific glutamate receptor–mediated effects on ChIs is unknown. However, it is worth noting that a high NMDA/AMPA receptor ratio was found at thalamic synapses formed by Pf terminals in rat MSNs.\textsuperscript{261, 262}

At the ultrastructural level, both group-I metabotropic glutamate receptors (mGluRs) (i.e., mGluR1 and mGluR5) are expressed extrasynaptically, or at the edges of glutamatergic synapses, on ChAT-labeled dendrites in the primate and rat accumbal core and shell regions,\textsuperscript{263} in agreement with data from the rodent dorsal striatum.\textsuperscript{264} Although direct evidence must be provided, the extrasynaptic glutamate spillover from cortical or thalamic terminals and/or the astrocytic release of glutamate are the most likely sources of activation of these receptors.\textsuperscript{265}

### GABAergic regulation of cholinergic interneurons

#### Striatal projection neurons: role of GABA and neuropeptides

Although the source(s) of GABAergic inputs to ChIs remains to be fully characterized, we have recently demonstrated that axon collaterals of GABAergic projection neurons provide major synaptic inputs to ChIs in the monkey putamen (Fig. 5).\textsuperscript{134} As much as one-third of intrastriatal GABAergic terminals from axon collaterals of direct-pathway neurons (Fig. 5B) and one-half of those from indirect-pathway neurons (Fig. 5C) form symmetric synapses with monkey ChIs.\textsuperscript{134} These findings are in striking contrast with rat data showing that ChIs receive GABAergic inputs from direct-, but not indirect-, pathway neurons in the dorsal\textsuperscript{266, 267} and ventral\textsuperscript{268} striatum.

In addition to GABA, the release of neuropeptides from axon collaterals of MSNs may also affect striatal cholinergic activity. Dorsal striatal ChIs display mRNA and protein expression for μ-opioid (daytime sensitive and mainly in the striosomes and the ventromedial caudate–putamen complex) and δ-opioid receptors in rats,\textsuperscript{269–271} as well as neurokinin (NK1) receptors in rats and humans.\textsuperscript{272–277} The cell bodies and dendritic processes of ChIs in the rodent accumbens shell also express μ- and δ-opioid receptor and NK1 receptor immunoreactivity.\textsuperscript{208, 271, 277–279} At the functional level, SP and Enk exert opposite effects on striatal ChI activity in rodents. While the striatal release or bath application of SP depolarizes ChIs and increases acetylcholine release in the dorsal striatum,\textsuperscript{277, 280–283} opposite effects are elicited following the endogenous Enk-mediated activation or \textit{in vitro} administration of μ- or δ-opioid receptors in the dorsal\textsuperscript{284–290} and ventral\textsuperscript{291, 292} striatum. Furthermore, a recent study revealed that μ-opioid receptor activation inhibits the \textit{in vitro} spontaneous activity of ChIs in the mouse dorsal striatum (functional territory unknown), independently of GABA(A) or NMDA receptor activation.\textsuperscript{293} The systemic administration of SP, however, decreases extracellular acetylcholine concentrations in the dorsal striatum and accumbens of freely moving rats,\textsuperscript{294} but most likely through the activation of
multisynaptic pathways. At the level of the ventral striatum, in vitro activation of μ- and δ-opioid receptors decreases acetylcholine release, similar to effects described in the caudate–putamen complex, and increases putative ChI responses to contingent Pavlovian training in rodents.

Altogether, it appears that ChIs in the dorsal striatum are under tight regulation by intrastratal GABAergic afferents that originate, in large part, from axon collaterals of GABAergic direct and indirect striatofugal neurons in primates (Figs. 5 and 6). In both primates and non-primates, the neuropeptides ENK and SP, which co-exist with GABA in MSN axon collaterals, also regulate ChI activity. Further studies must be performed to determine if this peptidergic modulation occurs in concert or in parallel with that mediated by the GABAergic system.

**Other sources of GABAergic inputs to Chls**—GABAergic afferents from various populations of striatal interneurons, the globus pallidus, and the VTA (mainly to the nucleus accumbens) may also contribute to the GABAergic regulation of Chls (Fig. 6), thereby providing additional substrates through which various sources of GABA may regulate striatal ChI activity.

**GABA receptors on Chls**—Consistent with their strong GABAergic synaptic innervation, ChIs in the dorsal striatum are enriched in both GABA(A) and GABA(B) receptor subunits in rats and primates, and electrophysiological evidence from in vitro and in vivo preparations in rodents have demonstrated that activation of either receptor subtype elicits inhibitory synaptic responses in ChIs and reduces in vivo acetylcholine release. However, conflicting results have been published about the GABAergic regulation of Chls in the rodent ventral striatum. In rodent accumbal slices, GABA application alters acetylcholine output and turnover, but not as strongly as in the caudate–putamen complex. On the other hand, the local application of GABA and GABA(A) or GABA(B) receptor agonists resulted in extracellular in vivo acetylcholine release in the accumbens of freely moving rats. A recent in vitro slice study demonstrated that high-frequency intra-accumbal stimulation produces GABA(B)-mediated TAN inhibition in rats. Overall, it appears that accumbal Chls are endowed with GABA receptors and display functional properties necessary for GABAergic modulation, although the functional context and mechanisms by which these inhibitory networks are recruited and involved in the regulation of accumbal Chls activity remain to be characterized.

**Dopaminergic regulation of cholinergic interneurons**

A substantial amount of pharmacological, electrophysiological, and neurochemical data has suggested close functional interactions between the mesostriatal dopaminergic systems and striatal ChIs in normal and diseased states. However, the exact synaptic mechanisms that mediate these interactions are complex and remain poorly understood. At the ultrastructural level, Kubota et al. showed that ChAT-immunoreactive soma and proximal dendrites receive direct synaptic inputs from TH-labeled terminals in the rat caudate putamen, while other studies in both the dorsal and ventral striatum of rodents
demonstrated close appositions with only scarce direct synaptic connections between putative dopaminergic terminals and ChIs.\textsuperscript{224, 310-315}

Despite this paucity of synaptic contacts, it is clear that ChIs activity is highly sensitive to dopamine receptor modulation, albeit to varying degrees between the dorsal and ventral striatum. In both striatal regions, D2 dopamine receptor mRNA is strongly expressed in ChIs,\textsuperscript{20, 316-318} with the greatest densities found in the dorsolateral caudate–putamen complex of rodents.\textsuperscript{319} The majority of ChIs in the dorsal and ventral striatum also express moderate to high levels of D5 dopamine receptors.\textsuperscript{20, 297, 319-323} On the other hand, D1 dopamine receptors are found on ChIs in the ventral, but not the dorsal, striatum in primates.\textsuperscript{317, 321} Although D3 dopamine receptor expression is undetectable in ChIs of the rodent dorsal striatum,\textsuperscript{297} ChIs in the ventral striatum, particularly those in the insula major of Calleja of the nucleus accumbens, are enriched in this dopamine receptor subtype.\textsuperscript{324-327} Thus, despite limited direct synaptic connections between dopamine terminals and ChIs, the strong dopamine receptor expression of ChIs allows them to be highly sensitive to extracellular dopamine.

In rodents and monkeys, \textit{in vitro} and \textit{in vivo} electrophysiological and pharmacological studies have revealed that cholinergic activity in both the dorsal and ventral striatum is significantly altered by manipulation of the midbrain dopaminergic systems, resulting in a wide range of receptor subtype–specific responses (i.e., no effect, increased or decreased cholinergic activity).\textsuperscript{11, 14, 19, 21, 22, 174, 217, 297, 328-335} Additionally, dopamine modulates cholinergic function in the caudate–putamen complex by regulating intrinsic cellular properties of ChIs and their synaptic afferents, particularly those from the glutamatergic corticostriatal system.\textsuperscript{19, 150, 161, 328, 336-340} Although such interactions most likely exist throughout the whole striatum, they have been mainly studied in dorsal striatal regions.

Functions of the intrastratal cholinergic network in the dorsal striatum and nucleus accumbens are significantly altered following acute or chronic chemical disruption of dopaminergic inputs from the SNc\textsuperscript{11, 13, 15, 19} or the VTA,\textsuperscript{16, 38} respectively. Thus, it is reasonable to conclude that the ascending dopaminergic systems are major regulators of ChIs activity, but that such control is largely mediated by diffusion of non-synaptic dopamine that can exert opposing and/or synergistic effects through the activation of specific dopamine receptors (D2, D3, and D5) expressed by ChIs, as well as through the manipulation of ChI synaptic afferents and intrinsic conductances.\textsuperscript{341-344}

### Cholinergic regulation of striatal activity

#### Cholinergic receptor expression in the dorsal and ventral striatum

**Muscarinic receptor expression of striatal neurons**—ChIs modulate the activity of striatal neurons through direct and indirect mechanisms via a wide range of pre- and postsynaptic cholinergic receptors.\textsuperscript{14, 251, 345} The G protein–coupled muscarinic cholinergic receptors (mACHRs) and ionotropic nicotinic cholinergic receptors (nAChRs) are located on the surface of striatal neurons and their various synaptic afferents.\textsuperscript{344-350} Five types of mACHRs have been genetically identified (M1–M5) and categorized into two groups on the basis of their distinct pharmacological properties upon activation: the \(G_{q/11}\)-coupled M1-like
receptors (M1, M3, and M5) that enhance internal calcium release through stimulation of phospholipases, and the $G_{i/o}$-coupled M2-like receptors (M2 and M4) that block calcium channel activity by reducing cyclic AMP formation through the inhibition of adenylyl cyclase.\textsuperscript{349, 351, 352}

The highly heterogeneous distributions of M1–M5 receptor mRNA, protein, and binding sites in the dorsal and ventral striatum, and within single striatal cell populations, contribute to the multifaceted distinguishing features of the striatal cholinergic system. The patterns of striatal mAChR expression have been studied using autoradiography, reverse transcription-polymerase chain reactions (RT-PCR), \textit{in situ} hybridization, and immunohistochemistry.\textsuperscript{353–367} Significant M1 mAChR mRNA and binding sites are heterogeneously expressed (i.e., patchy appearance) throughout the dorsal and ventral striatum of rats\textsuperscript{359, 360, 364, 366, 368} and primates\textsuperscript{354, 357, 362, 367}. The striosomes in the caudate nucleus of cats, monkeys, and humans display the highest level of M1 receptor binding, while the lowest striatal expression is found in the insula major of Calleja.\textsuperscript{353, 354} M2 receptor mRNAs are moderately expressed in the dorsal regions of the caudate–putamen complex\textsuperscript{369} and the core of the accumbens\textsuperscript{366} in adult rats. In cats and primates,\textsuperscript{362, 365, 370} M2 expression in the dorsal striatum is comparable to that in rodents,\textsuperscript{369} but the medial shell of the accumbens and the insula major of Calleja in primates also contain dense M2 receptor binding sites.\textsuperscript{354} The expression pattern of striatal M3 receptor mRNA and binding sites significantly differs from that of M1 and M2 receptors (i.e., they are mainly enriched in the mid-ventral and ventral regions of the dorsal striatum in rats and monkeys)\textsuperscript{362, 365, 370} and in the core and medial shell of the rodent accumbens.\textsuperscript{365, 370} The M4 mAChRs mRNA and binding sites are prevalent in all regions of the rodent striatum,\textsuperscript{360} although some studies have shown lower levels of M4 mRNA in the nucleus accumbens of rodents.\textsuperscript{359, 364} In rats, the expression of M4 receptor mRNA and protein exhibits a dense, patchy-like pattern (not clear if correlated to striosomal boundaries) in the caudate–putamen complex,\textsuperscript{359, 371, 372} but a more homogeneous expression in the nucleus accumbens.\textsuperscript{373, 374} Although displacement radioligand binding experiments have illustrated the likeliness of M3 and M4 receptor expression in the primate caudate nucleus and putamen,\textsuperscript{362, 367} a full characterization of these expression patterns using more specific markers must be established in the dorsal and ventral striatum of nonhuman and human primates.

At the cellular level, M1 mAChR mRNA is highly expressed by direct- (labeled with SP or D1 dopamine receptors) and indirect- (labeled with Enk or D2 dopamine receptors) pathway MSNs, neuropeptide Y (NPY)/somatostatin–containing interneurons, and ChIs in the dorsal striatum.\textsuperscript{375–382} A moderate number of Enk-containing projection neurons\textsuperscript{359, 375, 378} and ChAT-labeled neurons\textsuperscript{376, 380, 383} also contain M4 mAChRs. It is noteworthy that SP/D1 direct-pathway MSNs display a five-fold higher M4 mRNA expression level than that in Enk-labeled indirect-pathway neurons in rats.\textsuperscript{375, 378, 384} At the light microscopic level, M2 mAChRs are expressed in ChAT-labeled neurons and NPY/NADPH–containing interneurons in the rodent and monkey dorsal striatum.\textsuperscript{372, 375, 377, 385–387} Single-cell RT-PCR findings have suggested that mRNA for M3 receptors and the neuropeptide Enk coincide within around 10% of the same neurons in the rat dorsal striatum.\textsuperscript{378} Comparative data for M1, M2, M3, and M4 expression in specific cell populations are currently
unavailable for the nucleus accumbens of all species, as well as for the primate dorsal striatum.

With regard to M5 mAChRs, immunocytochemical and in situ hybridization studies have shown minimal staining in the striatum, most likely due to the low, undetectable level of M5 receptor mRNA expression in striatal neurons and the lack of sensitive M5 receptor antibodies to detect immunolabeling in striatal neurons and their afferents. Interestingly, activation of M5 receptors in the striatum inhibits dopamine release, while M5 receptor stimulation at the cell body level in the SNc increases the firing activity of dopaminergic neurons (see below for details).

**Nicotinic receptor expression in striatal neurons**—Nicotinic receptors form pentameric ion (Na⁺, K⁺, and Ca²⁺) channels that consist of either homomeric (α7 in the mammalian brain) or heteromeric combinations of α (α2–α10) and β (β2–β4) subunits, resulting in functional receptors with highly diverse pharmacological and functional properties. The α4 and β2 mRNA and protein are the predominant subunits expressed in the dorsal and ventral striatum of rodents and primates. In general, a similar homogeneous distribution pattern for nAChR subunit mRNA exists in the dorsal striatum of rodents and primates. However, the expression patterns for α4 and α7 mRNA are different from each other in the monkey and human dorsal striatum, but similar in rats. In addition, the α5, α6, and β3 subunits are more strongly expressed in the putamen than in the caudate nucleus of primates. At the cellular level, α7 and β2 nAChR subunits are co-expressed on ChIs and GABAergic axons of unknown sources in the rat dorsal striatum. As specific antibodies, receptor ligands, and pharmacological drugs are developed, a clear map of the cellular localization of nAChRs will be established across different neuronal populations, striatal regions, and species.

**Muscarinic and nicotinic receptor expression in striatal afferents**—Although there is no detailed quantitative assessment of mAChRs expression in striatal afferents, qualitative ultrastructural findings have suggested that putative glutamatergic terminals from either the cerebral cortex or thalamus express M1, M2, M3, and/or M4 mAChRs in the dorsal striatum. On the other hand, M5 receptors, which occasionally colocalize with α4β2 nicotinic receptors, are mainly expressed in midbrain dopaminergic axon/terminal processes. These findings are consistent with the expression of M5 receptor mRNA in SNc and VTA dopaminergic neurons and the physiological effects of M5 allosteric modulators on striatal dopamine release.

Several populations of striatal afferents express nicotinic receptors. Attempts at characterizing nAChR expression in striatal dopaminergic axons/terminals have been ongoing for decades by means of in situ hybridization, autoradiography, and immunoprecipitation techniques in the SNc, VTA, and striatum, along with striatal dopamine release studies in receptor subunit knock-out mice. Currently, the general consensus is that α3 (mainly in primates), α4, α5, α6, β2, and β3 subunits are expressed in highly diverse and complex subunit compositions on dopaminergic axons/terminals in the striatum. At the ultrastructural level, one study utilizing
double-label electron microscopy has revealed the co-expression of TH and β2 nicotinic receptors on dopamine terminals in the rat dorsal striatum. On the other hand, a combination of immunoprecipitation and radioligand binding studies performed on the monkey dorsal striatum have suggested that non-dopaminergic axons, possibly of glutamatergic origin, express nicotinic receptors with α7 subunits. Double-label confocal microscopy has demonstrated the co-expression of 5-hydroxytryptamine (5HT3) serotonin receptors and α4 nAChRs on terminals of an unknown chemical phenotype in the rat dorsal striatum. Nicotinic receptor expression on striatal (especially accumbal) afferents and their postsynaptic targets remains to be fully characterized in primates and non-primates.

**Electron microscopic localization of cholinergic receptors**—Electron microscopic studies of cholinergic receptor localization have only been carried out in the caudate–putamen complex of rats and mice and the monkey caudate nucleus. According to these studies, spines are the main striatal neuronal elements that express M1 mAChRs in rats and monkeys. With regard to presynaptic localization, M1 mAChRs are located on terminals forming asymmetric or symmetric synapses in the monkey dorsal striatum, while in rats, only terminal boutons that form asymmetric synapses express the M1 receptor subtype. By means of double-labeling experiments at the light microscopic level, M1 receptor expression has been found in calbindin-labeled, but not PV- or NADPH-labeled neurons, suggesting a preferential M1 expression in MSNs over GABAergic interneurons.

On the other hand, striatal M2 mAChRs are expressed on soma, dendritic shafts, and terminals that typically form symmetric axodendritic and axospinous synapses in rats and monkeys. Interestingly, M2 receptor immunoreactivity is also found at putative dendrodendritic synapses. In the rodent dorsal striatum, M3 receptors are located on spines and, like M4 receptors, on terminals that formed asymmetric axospinous synapses. In normal states, the plasma membrane of ChAT-labeled soma and dendrites displays immunoreactivity for M2 mAChRs, whereas M4 receptor immunostaining is mainly found intracellularly in the endoplasmic reticulum of ChIs or extrasynaptically on the plasma membrane of striatal MSNs. After acute oxotremorine (i.e., a nonselective muscarinic agonist) treatment, M2 receptors are trafficked from the plasma membrane of ChAT-labeled neurons to internally located endosomes, while M4 receptor expression is unaffected, suggesting different sensitivities and trafficking properties of these two receptor subtypes in response to increase cholinergic activation.

In line with evidence for presynaptic nAChRs in various brain regions, extrasynaptic β2 subunit immunoreactivity is found in TH-labeled nigrostriatal terminals forming axospinous symmetric synapses and in unlabeled terminals forming symmetric or asymmetric synapses with dendritic shafts in the rat dorsal striatum. Additional ultrastructural studies are needed to characterize the subcellular and subsynaptic location of other subunits of nicotinic receptors in the dorsal and ventral striatum of rodents and primates.
Cholinergic regulation of striatal neurons and their afferents

Autoregulation of cholinergic excitability in the striatum—Spike firing by ChIs sets a regulatory tone in the dorsal striatum through the tonic release of acetylcholine, acting on diverse muscarinic and nicotinic receptors located on striatal GABAergic neurons and their afferents. Additionally, cholinergic receptors influence endogenous cholinergic activity through both muscarinic autoreceptors (M2, M4) on ChIs themselves and nicotinic receptors on non-cholinergic synaptic inputs to ChIs. However, because of the limited availability of specific pharmacological tools and detailed assessments of cholinergic receptor expression, significant controversy exists about the relative roles of mAChRs and nAChRs in their autoregulatory function of ChIs in the dorsal striatum.

Information about the cholinergic receptor subtypes that regulate ChIs activity in the nucleus accumbens is minimal. However, the fact that low M2, but significant M4, receptor expression is found in the nucleus accumbens suggests a different role for the muscarinic receptor modulation of ChIs between the dorsal and ventral striatum.

Nicotine receptor modulation of ChIs has been reported in the dorsal and ventral striatum of rodents. In slices from the caudate–putamen complex, ChIs inhibit their own activity through nicotinic receptor–mediated activation of their GABAergic afferents (i.e., neurogliaform/NPY–containing interneurons and other GABAergic striatal neurons), resulting in a synchronized pause in cholinergic activity. Similarly, cholinergic tone in accumbal slices is also regulated by nAChRs, most likely located presynaptically on GABAergic afferents to ChIs.

Muscarinic and nicotinic modulation of GABAergic striatal neurons—Because of their diverse G protein coupling, multifarious neuronal and synaptic expression, and close proximity to nAChRs, acetylcholine-induced activation of mAChRs facilitates or suppresses the activity of striatal neurons. In striatal slices, the general activation of mAChRs by oxotremorine or carbachol decreases spontaneous inhibitory postsynaptic current (sIPSC) frequency and amplitude in MSNs of the caudate–putamen complex or nucleus accumbens in rodents. M1 receptor activation directly contributes to MSN depolarization and dendritic excitability through the coordinated modulation of calcium and potassium channels, or indirectly by regulating the suppression of the endocannabinoid system in the rodent dorsal and ventral striatum. On the other hand, MSN inhibition is also indirectly mediated through the M2- or M4-induced suppression of neurotransmitter release from cholinergic and/or glutamatergic terminals, and for the case of M4, similar effects were found for both striatonigral and striatopallidal MSNs. M4 receptor signaling was also shown to shape the firing activity and up- and down-state transitions of MSNs. In contrast to M4 regulation of both MSN populations, M1 receptor activation modulates the basal dendritic excitability of striatopallidal, but not striatonigral, neurons via the downregulation of their Kir2 potassium channels, or by the facilitation of their dopamine/DARP-32 signaling, in genetically-modified mice. At this time, it is unknown whether these concepts uphold in the primate dorsal and ventral striatum.
On the contrary to muscarinic receptors, there is limited evidence for nAChR expression in MSNs, suggesting that MSN excitability undergoes indirect nicotinic regulation through presynaptic receptors on GABAergic, glutamatergic, serotonergic, and/or dopaminergic striatal afferents.\textsuperscript{199, 345, 349, 409, 423, 443, 444, 452–454} In patch-clamp recordings from rodent caudate–putamen slices, nicotinic receptor agonists directly activate GABAergic interneurons that express PV, TH, or NPY (neurogliaform and non-neurogliaform), but not D1- or D2-containing MSNs.\textsuperscript{409} However, in this particular environment, only the GABAergic responses induced in MSNs by neurogliaform/NPY– and TH-containing interneurons respond to nAChRs (i.e., carbachol plus atropine).\textsuperscript{409} In whole-cell recordings from rat dorsal striatal slices, acetylcholine has a dual effect on fast-spiking (putative PV-containing) interneurons (FSIs). On one hand, it depolarizes FSIs by acting on non-desensitizing somatodendritic nAChRs, while it attenuates FSI-mediated GABAergic inhibition through activation of presynaptic mACHRs.\textsuperscript{345} Collectively, these findings suggest that in vitro muscarinic modulation may overpower nicotinic regulation of PV-containing interneurons in the dorsal striatum of rats, an effect that could be dependent on the basal ACh levels and firing activity of ChIs at the time of recordings.\textsuperscript{345}

Acetylcholine also modulates GABA release in the striatum through complex interactions between presynaptic mACHRs and nAChRs on the same GABAergic terminals.\textsuperscript{199, 409, 440, 442, 455} In synaptosomes from the dorsal striatum, atropine and the presumed M4 antagonist MT3 facilitate depolarization-evoked release of GABA, likely from presynaptic terminals.\textsuperscript{440, 442} On the other hand, nicotine and a variety of nAChR agonists evoke GABA release in striatal synaptosomes (and in other experimental configurations) that could be counteracted by muscarinic activation and/or antagonism of α\textsubscript{4}β\textsubscript{2} nicotinic receptor subunits.\textsuperscript{407, 442} On the basis of these observations, M4 mACHRs and α\textsubscript{4}β\textsubscript{2} nAChRs are thought to co-exist on the same GABAergic terminals (of unknown origin) in the dorsal striatum.\textsuperscript{442} As specific drugs for cholinergic receptor subtypes are developed and techniques optimized, the existence of similar types of interactions between other muscarinic and nicotinic receptors, as well as the chemical phenotype and source(s) of the terminals on which they reside, will be determined in the dorsal and ventral striatum.

**Muscarinic and nicotinic modulation of striatal glutamatergic transmission**—Cholinergic modulation of glutamatergic transmission at excitatory synapses is mediated through the activation of M1–M4 mACHRs in the rodent dorsal striatum. Muscarine and a putative M1 receptor agonist enhance NMDA (but not AMPA) receptor–induced depolarization of MSNs through postsynaptic mechanisms.\textsuperscript{451, 456} On the other hand, presynaptic M2/M3 mACHR activation decreases the probability of multivesicular release from glutamatergic terminals in striatal slices, thereby reducing corticostriatal glutamatergic transmission and the induction phase of long-term potentiation (LTP) in MSNs.\textsuperscript{457, 458}

Likewise, cholinergic single spikes depress cortically evoked excitatory postsynaptic currents (EPSCs) in one-third of MSNs located within 100 μm of the spiking ChI,\textsuperscript{459} while oxotremorine or a burst in ChI firing (induced by thalamic activation) inhibits both corticostriatal and thalamostriatal excitation of D1- and D2-expresssing MSNs.\textsuperscript{239} These data suggest that striatal ChIs finely regulate glutamatergic transmission at cortical and thalamic synapses on MSNs through temporal release of acetylcholine and activation of...
different pre- and postsynaptic mAChR subtypes. Presynaptic nAChRs activation also intricately modulates glutamate release from excitatory synapses, which often coincides with nigrostriatal dopamine release in the rodent dorsal striatum.\(^\text{340, 423, 460–462}\)

In the nucleus accumbens, muscarine application inhibits glutamate release, possibly via M3 receptors, in rat brain slices.\(^\text{455}\) In addition, general activation of mAChRs by oxotremorine reduces evoked EPSCs in MSNs, likely through the presynaptic inhibition of excitatory synapses.\(^\text{199}\) With the development of highly selective cholinergic receptor drugs, the mAChRs and nAChRs underlying cholinergic regulation of glutamatergic synapses in the nucleus accumbens will be delineated, along with those in the primate dorsal and ventral striatum.

**Muscarinic regulation of striatal dopamine transmission**—ChIs may directly and indirectly regulate dopaminergic transmission in the striatum through presynaptic activation of various subtypes of cholinergic receptors on dopaminergic, GABAergic, glutamatergic, and cholinergic axons.\(^\text{371, 372, 377, 463}\) There is evidence that this presynaptic regulation involves different cholinergic receptor subtypes in the dorsal versus ventral striatum.\(^\text{14, 19, 239, 377, 441, 446, 457, 459, 464}\) For example, muscarinic and nicotinic regulation of dopamine release in the rodent caudate–putamen complex requires M2/M4 mAChRs and nAChRs with \(\alpha_4\beta_2\) and \(\alpha_4\alpha_5\beta_2\) subunits, whereas M4 mAChRs and \(\alpha_6\beta_2\) nAChRs mediate these effects in the nucleus accumbens.\(^\text{14, 346, 465–468}\) These rodent data are at odds with nonhuman primate studies that show a strong \(\alpha_6\beta_2\) and \(\alpha_3\beta_2\) nicotinic receptor–mediated modulation of dopamine release in the monkey dorsal striatum.\(^\text{406, 467, 469, 470}\)

Findings from slices of muscarinic receptor knock-out mice have shown that acetylcholine-mediated (i.e., oxotremorine-induced) dopamine release is enhanced by activation of M4 mAChRs, but inhibited by M3 receptor activation.\(^\text{374, 411}\) M4 mAChRs likely mediate their effects through the regulation of neurotransmitter release from intrastriatal GABAergic terminals.\(^\text{374, 411}\) On the other hand, on the basis of data showing that M3 mAChRs are expressed in terminals forming asymmetric synapses (i.e., putatively glutamatergic),\(^\text{371, 372}\) it is unclear how these receptors inhibit dopamine release. Interestingly, although strong anatomical evidence is still needed, a recent study using fast-scan cyclic voltammetry has revealed that M5 receptor potentiation with a positive allosteric modulator (PAM) inhibits dopamine release in the rat dorsal striatum, opposing its excitatory effects when applied at the level of SNC neuronal cell bodies.\(^\text{415}\) Dopamine release in the caudate–putamen complex and nucleus accumbens is also indirectly regulated by activation of the pedunculopontine (PPN) and laterodorsal (LDT) tegmental nucleus, respectively, by means of ACh activation of mAChRs on dopaminergic neurons in the SNC and VTA. M5-mediated excitation of dopamine neurons in the SNC and VTA and/or\(^\text{471–475}\) M3-mediated inhibition of dopamine release from the SNC have been suggested as mechanisms underlying these effects.\(^\text{415, 472, 475}\)

**Nicotinic regulation of striatal dopamine transmission**—The nicotinic regulation of mesostriatal dopaminergic systems is altered in multiple neurological and psychiatric disorders.\(^\text{14, 180, 239, 346, 347, 391, 463, 476–481}\) Recent evidence indicates that nAChRs regulate striatal dopamine release by acting as “presynaptic filters.”\(^\text{463}\) They utilize both dopamine
and cholinergic neuron spiking activity (i.e., frequency and pattern) to adjust the probability of neurotransmitter release from dopaminergic axons/terminals in the striatum, independently from action potentials at dopaminergic soma. For example, in vitro optogenetic stimulation of ChIs in the dorsolateral striatum, or of their thalamostriatal afferents, triggers nAChR-mediated, frequency-independent (i.e., single action potentials or synchronized activity of ChIs) dopamine release from dopaminergic axons severed from their parent cell bodies. Thus, synchronized ChI-driven activation of dopaminergic axons, but not dopamine neuron firing, was proposed to mediate the role of dopamine in salience- or attention-related events.

The nicotinic modulation of striatal dopamine release driven by dopamine neuron activity is frequency-dependent and differs between striatal regions in rodents and monkeys. Because of their higher probability of dopamine release in response to a single stimulus, dopaminergic terminals in the dorsolateral striatum respond more robustly (i.e., release more neurotransmitter) to an arriving single action potential than those in the accumbens shell. In both regions, dopamine release is suppressed by nicotinic (partially, β2) receptor antagonism and/or desensitization. However, when dopamine release is induced by burst stimulations or chronic nicotine administration, the effect is seen only in the ventral putamen. Thus, these findings demonstrate that nicotinic modulation of dopamine release is dependent on the striatal subregion and dopaminergic firing frequency in the primate striatum, as observed in the rodent dorsal striatum and nucleus accumbens.

**Nicotinic modulation of striatal serotonin release**—In the rat dorsal striatum, confounding data have been reported about the nicotinic receptor–mediated regulation of serotonin release. In general, the activation of colocalized α4 (but not α3 and α5) nAChR and 5HT3 receptors increases presynaptic intracellular calcium in rat striatal synaptosomes, indicating possible modulation of neurotransmitter release and/or other presynaptic events by both nicotinic and serotonergic receptors located on the same terminals. In superfused rat slices and synaptosomes from the dorsal striatum, nicotine increases serotonin release in a dose-dependent manner, whereas locally applied acetylcholine or nicotine (but not mAChR agonists) decreases serotonin release in the feline caudate nucleus. Interestingly, in the latter study, the nicotine receptor–mediated effects on serotonin release are blocked by application of a GABA antagonist.
receptor antagonist, implying that these effects are perhaps indirectly mediated (via
unknown mechanisms) by striatal GABAergic interneurons, but not projection neurons. Although discrepancies exist between results of in vitro and in vivo studies, there is
agreement that serotonin release in the dorsal striatum is under modulation of the nicotinic
cholinergic system. It remains to be established whether this occurs in the primate dorsal
striatum, as well as in the rodent and primate nucleus accumbens.

**vGluT3 expression in Chls: role in cholinergic striatal regulation**—While
vGluT1 and 2 are commonly found in known glutamatergic neurons, such is not the case for
vGluT3 in the dorsal striatum. In ChAT-Cre mice, some striatal Chls have terminals
enriched in vGluT3, and activation of Chls mediates fast vGluT3-dependent glutamatergic transmission at synapses with FSIs. At the ultrastructural level, vGluT3 and vAChT co-exist on the surface of some synaptic vesicles, suggesting the possible co-release of glutamate and acetylcholine from single vesicles. In line with these observations, the disruption of the vGluT3 gene causes a hypocholinergic striatal phenotype. The significance of these findings in the primate striatum remains to be established.

**Cholinergic systems in transgenic ChAT-Cre mice**—It is noteworthy that several of
the aforementioned studies used optogenetic methods in dorsal and ventral striatal slices of
ChAT-Cre and ChAT-ChR2-EYFP Bac transgenic mice. However, the recent
evidence of vesicular acetylcholine transporter (vAChT) overexpression, the subsequent
amplified cholinergic tone and behavioral deficits (i.e., amplified drug-induced stereotypies), and the decreased density of ChAT-labeled neurons and neuropil in these
mice, raise caution about data interpretation and translation from these animals to
primates. More information should be gathered from other transgenic mice Cre lines to
determine if the baseline transmission of the neurotransmitter system under study is altered
in these animals.

**Striatal cholinergic system dysfunction in Parkinson’s disease and cocaine
drug addiction**

Dysfunction of the nigrostriatal and mesolimbic dopaminergic systems prompts a number of
neurochemical, physiological, and behavioral changes in basal ganglia disorders, such as PD
and drug dependency. However, in contrast to the significant dopaminergic nigrostriatal degeneration in PD, stimulant addiction partly depends on hyperdopaminergic functions that “hijack” normal learning processes to reinforce their own acquisition,
gradually leading to habitual drug seeking, loss of self-control, and relapse
susceptibility. At the circuit level, both disorders manifest abnormal
dopaminergic regulation of striatal principal neurons, intrastriatal circuits, and striatal
afferents.

The abnormal activity of multiple neurotransmitter systems, including the cholinergic
systems, precedes or coincides with dopaminergic dysfunction in parkinsonism and drug
addiction, suggesting that a striatal dopamine–acetylcholine imbalance underlies some aspects of the pathophysiology of parkinsonism and substance
However, the time point and mechanisms of this cholinergic dysfunction remain poorly understood, and the development of new therapeutic approaches aimed at cholinergic systems is limited for these disorders. Contributing factors to this shortfall likely result from the complex and diverse regulatory functions, physiological properties, and behavioral correlates of striatal ChIs discussed above, along with the limited availability of reliable and specific drugs to regulate cholinergic transmission. In the following discussion, we review and compare the changes to the striatal cholinergic systems that have been described in PD animal models (i.e., 6-hydroxydopamine (6-OHDA)–lesioned or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated rodents and monkeys) and in animals acutely or chronically exposed to cocaine. The suitability of cholinergic therapeutic interventions for PD and cocaine addiction/abuse will be considered. It is noteworthy that striatal cholinergic dysfunction has also been reported in many other brain disorders, such as Huntington’s disease, dystonia, Alzheimer’s disease).

**Striatal cholinergic dysfunction in Parkinson’s disease**

Because the complex interplay between the nigrostriatal dopamine and intrastriatal cholinergic transmission involves both direct and indirect routes that use various transmitter systems, the interpretation of changes in the dopamine–acetylcholine balance that occur in various animal models of PD, may be difficult. For example, although increased *in vitro* extracellular levels of acetylcholine and decreased AchE activity have been shown in the dorsal striatum of rodent and monkey PD models, respectively, neither the spontaneous firing rate of TANs (i.e., putative ChIs) or the level of synchronization of TAN activity is significantly altered in MPTP-treated monkeys. However, TANs display an increase in their oscillatory activity (~10–20 Hz), along with decreased sensory responsiveness to rewards, in the dopamine-denervated monkey dorsal striatum. In rodent models of PD, TANs exhibit an apparent reduced after-hyperpolarization current, possibly contributing to their reduced spike frequency adaptation under these conditions. It is not fully understood how these changes in cholinergic activity affect overall striatal function and output in parkinsonism, although a recent review considered the idea of whether an enhanced cholinergic tone is sufficient to sustain synchronous oscillatory activity of striatal projection neurons in the parkinsonian state.

Another layer of complexity revolves around the network connectivity of ChIs in the dorsal striatum. As mentioned above, ChIs express D2 and D5 dopamine receptors, and dopamine receptors become hypersensitive, often resulting in enhanced cholinergic responsiveness to their activation in the dopamine-depleted state. Thus, the altered inhibitory (via D2 receptors) and excitatory (via D5 receptors) actions of dopamine on ChIs activity might be partly responsible for striatal cholinergic changes in parkinsonism. Striatal ChIs also express receptors that are co-expressed with dopamine receptors and negatively affected in PD, such as the adenosine A2A receptors, which are currently the target of multiple clinical studies. Furthermore, anatomical and functional changes in non-dopaminergic afferents and efferents of ChIs must also be considered. For example, ChIs increase their connectivity with striatopallidal (D2-expressing) projection neurons, but decrease their interactions with striatonigral (D1-expressing) neurons after striatal dopamine.
depletion.\textsuperscript{531, 532} The abnormal activity of MSNs in the parkinsonian state\textsuperscript{533–535} may also indirectly contribute to changes in striatal cholinergic function\textsuperscript{536} because of the substantial connection between MSN axon collaterals and ChIs in the dorsal striatum.\textsuperscript{134} The substantial cell loss in the CM/Pf thalamic nuclei of MPTP-treated monkeys\textsuperscript{537} and PD patients\textsuperscript{538–541} and the subsequent degeneration of the thalamostriatal system, along with impaired GluN2D NMDA receptor signaling in parkinsonian rodents,\textsuperscript{542} might significantly impact the glutamatergic regulation of ChIs in parkinsonism.

Thus, the functional alterations of ChI activity in the dopamine-depleted state further highlight the importance of targeting the striatal cholinergic system in PD. However, a more complete description of the expression and function of specific cholinergic receptor subtypes in the striatum and other basal ganglia nuclei, as well as the plastic changes ChIs undergo in parkinsonism, are necessary to identify therapeutically relevant targets for motor and non-motor symptoms of PD. In addition, therapeutic agents that avoid the unwanted cognitive and autonomic side effects of the currently available, broad-spectrum cholinergic drugs, must be developed to achieve that goal.

**Striatal cholinergic dysfunction in cocaine addiction**

Although dopaminergic systems are antagonistically altered in PD and cocaine abuse, the self-administration of cocaine in rodents increases striatal acetylcholine release, as seen in parkinsonism. However, this effect occurs primarily in the accumbal shell (and to a lesser extent, in the core of the accumbens and the dorsal striatum), and is D1, but not D2 receptor dependent.\textsuperscript{331, 334, 543} In agreement, ChIs activation has also been reported in the accumbens shell and the ventromedial caudate putamen after acute self-administration of cocaine in rats.\textsuperscript{544} In contrast, ChIs ablation in the rodent nucleus accumbens enhances long-lasting behavioral changes, such as hyperlocomotion, associated with cocaine administration, whereas accumbal acetylcholine enhancement through AchE inhibition suppresses these cocaine-induced behaviors.\textsuperscript{545–547} Moreover, hypercholinergic rodent models of depression exhibit a reduction in their cocaine-induced responses to locomotion and dynorphin neuroadaptations.\textsuperscript{548} As recently described, the effects of cocaine abuse on cholinergic systems are highly dependent on physiological and experimental variables, such as receptor subtypes, brain region localization, drug dosage, form of administration (i.e., voluntary versus involuntary), length of use (i.e., acute versus chronic), and phase of addiction (i.e., initiation, reinforcement, withdrawal, and relapse),\textsuperscript{543, 549–553} providing possible explanations for the complex responses of the striatal cholinergic system to cocaine use. Further contribution to this complexity may be accounted for by the specific effects of dopamine on accumbal ChIs which, in contrast to dorsal striatal ChIs, also heavily express D3 dopamine receptors.\textsuperscript{324–327}

The role of the striatal cholinergic system in the reward responses induced by short- and long-term use of addictive substances, such as cocaine, has not been fully delineated at this time.\textsuperscript{308, 554, 555} However, data from the dorsal striatum revealed that TANs (putative ChIs) play a role in various aspects of reward behavior associated with goal-directed and habitual learning, reinforcement, and motivation\textsuperscript{19, 149, 164, 174, 179, 180, 187, 188, 556} through their actions on dopamine spike discharge and release.\textsuperscript{204, 481} Additionally, dorsal striatal ChIs

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respond to sensory cues associated with reward (and non-rewards) with an initial excitation and pause in activity, followed by a rebound excitation.\textsuperscript{19} In contrast, TANs in the core or shell of the nucleus accumbens respond to specific reward parameters with either an increase or decrease in firing activity, respectively.\textsuperscript{190, 208, 209} For instance, accumbal TANs display bidirectional reinforcement responses during reward learning (i.e., increased firing after reward appearance, but paused firing upon reward omission).\textsuperscript{205} Furthermore, nigral and ventral tegmental dopaminergic afferents to the striatum respond to both the reward itself and reward-predicted stimuli with a burst in their activity.\textsuperscript{180, 505} These studies strongly suggest that accumbal and dorsal striatal ChIs likely contribute to cocaine-induced reward responses.

Ensembles of accumbal neurons with different functional properties respond to natural and unnatural rewards (including cocaine) in a variety of ways. For example, distinct temporal firing patterns of striatal neuron populations were recorded in the rodent and monkey accumbens (and dorsal striatum) in response to both cocaine and water reinforcement, implying that cocaine utilizes a similar brain reward circuitry and temporal encoding as natural rewards.\textsuperscript{197, 556–559} Currently, it is unclear if cholinergic cells are represented in these various striatal neuron populations, but on the basis of the physiological properties of dorsal striatal ChIs, their well-characterized responses to reward, and their cocaine-induced inhibition in the dorsal striatum, it seems likely that they may be involved in these responses.\textsuperscript{164, 175, 179, 180, 187, 189, 239} In line with this possibility, recent data from the medial nucleus accumbens of BAC transgenic ChAT-Cre mice demonstrated that TAN photoinhibition during cocaine place conditioning (20 mg/kg) significantly decreased preference for the cocaine-affiliated chamber, but did not alter cocaine conditioning itself.\textsuperscript{201} Furthermore, acute cocaine administration increased the in vitro spontaneous activity of ChIs.\textsuperscript{201}

Electrophysiological recordings of ChIs in various sub-compartments of the nucleus accumbens must be performed to better define the firing properties of these neurons in the ventral striatum under normal conditions and after acute and chronic cocaine self-administration in rats and non-human primates. Additionally, the responses of ChIs to reward-associated cues and stimuli (unnatural versus natural) must be further characterized across subregions of the nucleus accumbens.\textsuperscript{560–562} Because neural activity changes throughout the entire striatum have been described in response to cocaine,\textsuperscript{563, 564} it may be useful for future studies to directly compare changes in TAN (putative ChIs) activity between the dorsal and ventral striatum, so that the participation of striatal TANs in the neural circuits of cocaine reward can be better understood.

**Cholinergic therapies for Parkinsonism and cocaine dependency: A major challenge?**

**Cholinergic therapy in Parkinson’s disease**

**Muscarinic receptor–related drugs:** Conflicting data from previous studies, along with the limited understanding of the dorsal and ventral striatal cholinergic systems and the lack of specific drugs, have hindered the progress of developing cholinergic drug therapies for the treatment of PD and drug dependency. Anti-cholinergic drugs, used alone or in conjunction with dopaminergic therapies, were utilized as one of the first treatments for PD,\textsuperscript{565–567}...
although their use was limited because of significant cognitive and autonomic side effects. More than a century later, the use of anti-cholinergic agents as PD therapeutics still remains a major challenge. Anticholinergic drugs are sometimes utilized as a supplement to dopamine-replacement therapy for PD to alleviate some of the non-motor and motor symptoms, such as bladder dysfunction and tremor. However, the long-term use of dopaminergic drugs that involves the progressive ramping up of dosages eventually leads to abnormal, involuntary movements (dyskinesias) and psychiatric complications in PD patients, while aversive cognitive and autonomic side effects are the consequences of the non-selectivity of current anti-cholinergic therapies. As a result, the search for selective cholinergic receptor drugs that regulate striatal cholinergic activity are currently being investigated as alternative monotherapies or combination therapies in conjunction with dopaminergic agonists for PD.

In that regard, selective M1 mAChR antagonists have weaker anti-parkinsonian properties than the non-selective muscarinic drugs. In mice, the genetic deletion of M1 mAChRs partially prevents the loss of glutamatergic innervation of MSN dendritic spines in models of PD, while M1 receptor antagonists or ChI ablation reduce L-DOPA–induced dyskinesias in mice with striatal dopaminergic denervation. However, their effects must be interpreted cautiously, because the M1 receptor antagonists used in these studies also partly inhibit M4 mAChRs. In fact, it may be more practical to regulate M2/M4 muscarinic autoreceptors of striatal ChIs to inhibit acetylcholine release from these neurons and thereby promote dopaminergic neurotransmission. On the other hand, because M4 is expressed postsynaptically in dendrites and spines of MSNs and presynaptically in glutamatergic striatal afferents, it could be beneficial to also regulate these interactions for the attenuation of PD symptoms. In agreement, studies have demonstrated that M4 mAChR mRNA expression and autoreceptor signaling that normally regulate ChIs spiking and acetylcholine release are significantly reduced in the dorsal striatum of dopamine-depleted rodents, further supporting the need for preclinical testing of these drugs in reliable animal models of PD. Thus, the development of highly specific M1- and M4-related drugs is necessary to directly test these hypotheses.

**Nicotinic receptor–related drugs:** Another potential cholinergic target for PD are the nAChRs expressed by striatal neurons (data inconclusive for ChIs) and nigrostriatal terminals. Drugs aimed at α4β2 and α6β2 nicotinic receptors, indeed, exposed potential neuroprotective (for midbrain dopaminergic neurons), anti-parkinsonian, and anti-dyskinetic effects in animal models of PD. Numerous studies have demonstrated a drastic loss of α6β2 nicotinic receptors (primarily responsible for acetylcholine-induced dopamine release in primates) in parkinsonian animal models and PD patients, along with a less severe reduction in α4β2 receptor subtypes. Because of their localization on dopaminergic terminals, striatal α4α6β2-expressing nAChRs are significantly reduced after nigrostriatal damage, leaving only α4β2- and α6β2-expressing non-dopaminergic neurons to regulate acetylcholine-induced dopamine release in the striatum of late-stage parkinsonism. In partially dopamine-depleted parkinsonian rodents and monkeys, nicotine synergistically acting on α4β2 and α6β2 nAChRs decrease abnormal, involuntary movements induced by commonly used dopaminergic therapies. Therefore,
PD therapy aimed at nAChRs could be beneficial, particularly if used as an early treatment strategy at a time when nicotinic agonists acting at the α6β2 (and possibly α4β2) subtype could still induce dopamine release from intact dopaminergic axons. Further electrophysiological and pharmacological studies using specific nicotinic-related drugs are needed to help clarify these uncertainties.

**Cholinergic therapy for cocaine addiction**—As for parkinsonism, studies of therapeutic interventions for stimulant addiction have revolved mainly around dopaminergic therapies, because of the compelling evidence that excessive dopamine release and abnormal mesolimbic activity occur with cocaine use and addiction. However, therapies aimed solely at the dopaminergic system in cocaine-addicted individuals were not found to be successful, possibly due to the fact that cocaine dependency affects diverse neurotransmitter systems beyond the dopaminergic mesostriatal projection. Due to the tight dopamine–acetylcholine relationship and abnormal cholinergic activity associated with cocaine abuse in the nucleus accumbens, research on possible cholinergic interventions has also been undertaken. As in PD, anti-cholinergic drugs were first assessed for their efficacy in alleviating stimulant addiction in animal models, on the basis of their potential to possibly reverse increased striatal acetylcholine release after cocaine use. However, because many anti-cholinergic drugs actually enhance the effects of cocaine, the effectiveness of cholinergic agonists/partial agonists on the abuse-related effects of cocaine are being examined.

The expression of M1-like and M2-like cholinergic receptors is either upregulated, downregulated, or unchanged in response to cocaine use, mirroring the complexity of D1-like and D2-like dopamine receptor changes observed with cocaine self-administration. On the basis that acetylcholine may enhance the abuse-related properties of cocaine, pharmacological studies first attempted to assess the effects of anti-muscarinic and anti-nicotinic drugs on cocaine abuse in animals. These studies revealed that mAChR antagonists dose-dependently decrease cocaine self-administration and reinstatement in rats and monkeys and that cocaine conditioned place preference is significantly reduced in M5 mAChR knock-out mice.

Similarly, nicotinic receptor blockade prevents the escalation of cocaine self-administration in rats with extended daily access, but does not block the actual drug intake. In humans, a nicotine receptor antagonist, mecamylamine, reduces cocaine-induced craving, supporting preclinical findings of decreased nicotine and cocaine self-administration in mice treated with this drug. The co-administration of α7 and β2 nicotinic receptor antagonists or mecamylamine also prevents the development of cocaine-induced increases in dopamine release on repetitive cocaine injections (i.e., sensitization) in mice. In addition, nicotine application in cocaine-dependent rats enhances cocaine-seeking behavior and potentiates cocaine reinforcement, while stimulating cue-elicited craving in humans.

Because the systemic administration of nonselective muscarinic antagonists enhances cocaine-induced effects, systemically-injected muscarinic agonists/partial agonists were also assessed for their ability to minimize cocaine dependency. Nonselective muscarinic agonists, such as oxotremorine and pilocarpine, xanomeline, as well as M1-
specific muscarinic agonists, block chronic cocaine self-administration and decrease cocaine discrimination in mice.\textsuperscript{608} Furthermore, an M4 positive allosteric agonist blocks cocaine-induced dopamine release in both the dorsal and ventral striatum of wild-type mice.\textsuperscript{609} However, dissimilar findings between the dorsal and ventral striatum were recently observed in studies examining cocaine-induced effects in M4 muscarinic receptor knock-out mice. Cocaine injections increase dopamine release in the nucleus accumbens, but not in the dorsal striatum, of mice with an M4 receptor gene deletion.\textsuperscript{609, 610} It was hypothesized that M2 receptors uniquely located on ChIs in the dorsal, but not the ventral, striatum compensate for changes in dopamine levels observed after cocaine injections in M4 receptor knock-out mice.\textsuperscript{609, 610} Thus, M4 receptors expressed by ChIs in the nucleus accumbens may have a greater role in regulating dopamine transmission than those in the dorsal striatum,\textsuperscript{576, 609} and striatal ChIs may regulate dopaminergic activity via M2 in the dorsal striatum but M4 in the ventral striatum.

Altogether, these unexpected neuroadaptations in the cholinergic system in response to cocaine use may possibly be due to the complex heterogeneity of the dopamine–acetylcholine interactions in the nucleus accumbens, along with different accumbal regions examined in these studies. Alternatively, ChIs may undergo plastic changes (i.e., increased synapses per neuron (including glutamatergic asymmetric synapses)) and larger dendritic branching, after repeated cocaine self-administration, which has been extensively demonstrated for spiny and aspiny neurons in the core and shell of the accumbens.\textsuperscript{611–614}

Although still poorly characterized, the potential for the use of cholinergic therapy in addiction remains of interest, particularly with the development of more selective muscarinic and nicotinic receptor agonists/antagonists.\textsuperscript{392, 393, 432, 573, 608, 615, 616} Specific positive allosteric modulators for M1, M2, M4, and M5 have now been developed and proven to be highly selective for their receptor subtypes.\textsuperscript{570, 571, 617, 618} The therapeutic assessment of these drugs alone, or in combination with dopaminergic receptor antagonists, should be explored in normal and cocaine-dependent animal models to further define the underlying roles and therapeutic relevance of specific cholinergic receptor subtypes in cocaine (or other psychostimulants) abuse and dependency.

**Concluding remarks**

In conclusion, the findings discussed in this review highlight the morphological heterogeneity of ChIs between species and functional regions of the striatum. Thus, in contrast to the common view that ChIs represent a functionally uniform population of neurons, \textit{in vivo} and \textit{in vitro} evidence clearly indicates that dorsal and ventral striatal regions contain different populations of ChIs. In addition to striking morphological differences, these neurons are differently regulated by extrinsic afferents and mediate some of their effects through different cholinergic receptor subtypes. Remarkably, striatal acetylcholine release increases in parkinsonism\textsuperscript{380, 518, 519} and after cocaine self-administration,\textsuperscript{331, 334, 543} although the dopaminergic mesostriatal system undergoes contrasting damage in these disorders. However, broad-spectrum anti-cholinergic drugs often induce negative side effects in parkinsonism and exacerbate the addictive behavioral properties of cocaine. Therefore, alternative therapeutic interventions that selectively target
muscarinic or nicotinic receptors and their receptor subunits may be more effective in providing some relief for these disorders. In addition, because other neurotransmitter systems, such as those that utilize glutamate, opioids, endocannabinoids, or neuropeptides, also undergo significant alterations in parkinsonism and stimulant addiction, the future development of drugs that preferentially target these non-dopaminergic systems could also allow for the indirect regulation of ChI activity in the dorsal and ventral striatum.

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Figure 1.
Morphological characterization of striatal cholinergic interneurons.
(A–G) Light micrographs of ChAT-positive interneurons in various dorsal and ventral striatal regions of rhesus monkeys. Note the differences in size and shape of labeled cell bodies, the extent of dendritic arborization, and intensity of neuropil immunostaining between the different sectors of the striatum. Scale bars = 200 μm in A (applies to B and C), in D (applies to E and F), and in G.
Figure 2.
Morphological differences of cholinergic interneurons between the rat and primate striatum. (A, B) Reconstructions of the somatodendritic domain of Golgi-impregnated ChAT-immunopositive neurons in the rat striatum; taken from Refs. 124 (A) and 85 (B). (C, D) Reconstruction of the somatodendritic domain of Golgi-impregnated AchE-positive neurons in the rat striatum; taken from Refs. 124 (C) and 127 (D–E). (F–H) Reconstructions of the somatodendritic domain of three Golgi-impregnated AchE-positive neurons in the human (F), rhesus monkey (G), and baboon (H) striatum (from Ref. 129). Note the smaller cell body size and less profuse dendritic arborization of cholinergic interneurons in the rat versus the primate striatum. Scale bars = 50 μm.
Figure 3.
ChAT immunolabeling in the monkey dorsal striatum.
(A–C) Low-power light micrographs of ChAT immunostaining through the rostrocaudal extent of the monkey striatum. Note the patchy neuropil staining in the anterior striatum (A) and the positive rostrocaudal gradient in the intensity of ChAT-immunoreactive neuropil (compare A with C). Ci depicts a higher-power view of ChAT-labeled cells bodies in the dorsal part of the postcommissural putamen (asterisk in C). Scale bars = 3 mm in AC; 600 μm in Ci. Abbreviations: Pre-Comm, precommissural; Comm, commissural; Post-Comm, postcommissural.
Figure 4.
ChAT immunolabeling in the monkey ventral striatum.
(A–F) Low-power views of ChAT immunoreactivity through the rostrocaudal extent of the monkey ventral striatum. The approximate interaural stereotaxic levels of each section are indicated in the lower right corner of the different micrographs. The insets show low-power views of the striatal sections from which the accumbens micrographs were taken (labeled with an asterisk). Note the highly heterogeneous ChAT-immunoreactive neuropil along the entire extent of the rostrocaudal axis of the nucleus accumbens. Scale bar = 700 μm in A; 900 μm in B (applies to C, E–H); 800 μm in D. Scale bars for insets = 3 mm.
Figure 5.
Summary of the known sources and relative abundance of synaptic inputs on ChAT-positive neurons in the monkey putamen. (A) Schematic of the different synaptic inputs to ChIs in the monkey putamen; taken from Ref. 134. Note that most synaptic inputs to ChIs are from putative GABAergic terminals (including SP$^+$ and ENK$^+$ terminals) that form symmetric synapses (i.e., blue and green terminals in panel A). Putative glutamatergic inputs are sparse and predominantly localized on the distal dendrites of ChIs. B–C show examples of SP$^-$ (B; + Ter) or ENK$^-$ (C; + Ter) positive terminals in contact with ChAT-positive dendrites in the monkey putamen. In the material, SP and ENK were localized with pre-embedding immunogold, while ChAT was labeled with immunoperoxidase. Scale bars: 0.5 μm. Abbreviations: +Ter, substance P$^-$ or enkephalin-immunoreactive terminals; U Ter, unlabeled terminals.
Figure 6.
Synaptic inputs to striatal cholinergic interneurons. Schematic showing the main intrastriatal synaptic connections of striatal cholinergic interneurons. Note that the thalamus, cerebral cortex, GPe, and SNc also contribute, to varying degrees, direct synaptic inputs to ChIs. Some striatal afferents are not depicted because of the lack of detailed knowledge of their synaptic connections with ChIs. Full lines indicate connections shown by electron microscopy and hatched lines indicate putative connections that remain to be confirmed at the electron microscopic level. Abbreviations: ACh, cholinergic interneurons; CT, calretinin-positive interneurons; Enk, enkephalin-positive MSNs; SP, substance P-positive MSNs; ST, somatostatin-positive interneurons; PV, parvalbumin-positive interneurons. See Ref. 134 for more detail.
### Table 1
Morphological and ultrastructural characteristics of ChIs in the dorsal and ventral striatum of rodents and primates

<table>
<thead>
<tr>
<th>ChI characteristics</th>
<th>Rodents</th>
<th>Primates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DS—morphology &amp; distribution</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soma size (diameter)</td>
<td>17–35 μm</td>
<td>35–50 μm</td>
</tr>
<tr>
<td>Soma shape</td>
<td>Mainly oval</td>
<td>Highly diverse</td>
</tr>
<tr>
<td>Dendritic tree size</td>
<td>Moderate, infrequently branched</td>
<td>Large, highly branched</td>
</tr>
<tr>
<td>Somata densities</td>
<td>Highest rostrally</td>
<td>Highest caudally</td>
</tr>
<tr>
<td>Neuropil densities</td>
<td>Mostly homogeneous</td>
<td>Moderately patchy</td>
</tr>
<tr>
<td>Ultrastructure</td>
<td>Indented nucleus, organelle-rich cytoplasm, subsurface cisternae, and lipofuscin granules</td>
<td>Indented nucleus, organelle-rich cytoplasm, subsurface cisternae, and lipofuscin granules</td>
</tr>
<tr>
<td><strong>NA—morphology &amp; distribution</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soma size (diameter)</td>
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<td>20–40 μm</td>
</tr>
<tr>
<td>Soma shape</td>
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<tr>
<td>Dendritic tree size</td>
<td>Moderate, infrequently branched</td>
<td>Moderate, infrequently branched</td>
</tr>
<tr>
<td>Somata densities</td>
<td>Highest medially</td>
<td>Highest medially and in insula major of Calleja</td>
</tr>
<tr>
<td>Neuropil densities</td>
<td>Moderately patchy</td>
<td>Extensively patchy</td>
</tr>
<tr>
<td>Ultrastructure</td>
<td>Indented nucleus, organelle-rich cytoplasm, subsurface cisternae, and lipofuscin granules</td>
<td>Not available</td>
</tr>
</tbody>
</table>

Abbreviations: DS, dorsal striatum; NA, nucleus accumbens