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REVIEW ARTICLE

Targeting the cholinergic system in Parkinson's disease

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Motor control in the striatum is an orchestra played by various neuronal populations. Loss of harmony due to dopamine deficiency is considered the primary pathological cause of the symptoms of Parkinson's disease (PD). Recent progress in experimental approaches has enabled us to examine the striatal circuitry in a much more comprehensive manner, not only reshaping our understanding of striatal functions in movement regulation but also leading to new opportunities for the development of therapeutic strategies for treating PD. In addition to dopaminergic innervation, giant aspiny cholinergic interneurons (ChIs) within the striatum have long been recognized as a critical node for balancing dopamine signaling and regulating movement. With the roles of ChIs in motor control further uncovered and more specific manipulations available, striatal ChIs and their corresponding receptors are emerging as new promising therapeutic targets for PD. This review summarizes recent progress in functional studies of striatal circuitry and discusses the translational implications of these new findings for the treatment of PD.

Keywords: Parkinson's disease; motor control; acetylcholine; dopamine; nicotinic receptor

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INTRODUCTION

PD is the second most common neurodegenerative disorder, affecting ~1%–2% of the world population over the age of 60 [1–3]. Patients typically suffer from involuntary tremors, muscle rigidity, and postural instability. These motor symptoms are believed to stem from an imbalance in the output of the striatum caused by a loss of nigrostriatal dopamine innervation [3, 4]. Despite decades of treatment efforts focusing on dopamine modulation, several lines of recent evidence have indicated that the striatal cholinergic system also plays an essential role in the information processing of the striatum and might emerge as a new drug target for treating PD. In this review, I will first update our current understanding of motor control in the striatum, highlighting the new insight into the role of dopamine in this process. I will then focus on recent progress in functional investigations of the striatal cholinergic system and discuss the implications of these new findings for therapeutic approaches of PD.

MOTOR CONTROL IN THE STRIATUM

The neural circuits in the striatum play a central role in motor planning and action selection. They are also the areas that are most affected by dopamine depletion in PD and the most critical therapeutic targets for treating the disease [5, 6]. The striatum receives excitatory innervations predominantly from the cortex and thalamus, and functions as a primary relay to other basal ganglia nuclei [7–10]. More than 90% of neurons in the striatum are medium spiny neurons (MSNs), which are GABAergic projection neurons that inhibit their targets when activated. MSNs do not exhibit spontaneous activity in vitro and tend to fire at ~1 Hz in behaving animals unless under significant transient afferent input. The remaining striatal neurons are mainly giant aspiny cholinergic interneurons (ChIs, 1%–3%) and GABAergic interneurons (2%–5%). GABAergic interneurons are local regulation neurons that can be subdivided into fast-spiking interneurons, calretinin-expressing interneurons, and low-threshold spiking interneurons. Although both use GABA as a neurotransmitter, GABAergic interneurons are distinct from MSNs in terms of morphology, projection, regulation, protein expression and firing activity [11].

The heart of the functional organization of the striatum is the so-called "direct/indirect pathway" model first proposed by Mahlon R Delong and his colleagues in the 1990s [12]. Roughly half of striatal MSNs express high levels of dopamine D1 receptors, forming the foundation of the direct pathway (also referred to as the striatonigral projection). The other half express dopamine D2 receptors and mainly innervate the pallidum, forming the indirect pathway (striatopallidal projection) [13–16]. This orthogonal organization of the motor control strategy is simple and seems to be remarkably conserved among all vertebrate species [17]. The canonical theory derived from multiple disciplines of studies postulates that the two distinct populations of MSNs, together with their corresponding pathways, might exert opposite roles in motor function, with direct pathway facilitating movement and indirect pathway suppressing it [18–22]. While many early observations reconciled with this working model, direct evidence was missing for a very long time until transgenic and optogenetic approaches that allowed for recruiting specific pathways became available [10, 23–26]. It was shown that specific activation of the direct pathway using channelrhodopsin-2, a light-sensitive ion channel that triggers firing in neurons, promotes locomotion while stimulating the indirect pathway increases freezing and impedes movement initiation [10, 14, 24, 27].

This simple rate model, in which activation of the direct pathway is prokinetic and activation of the indirect pathway is antikinetic, was recently challenged by the Costa laboratory.

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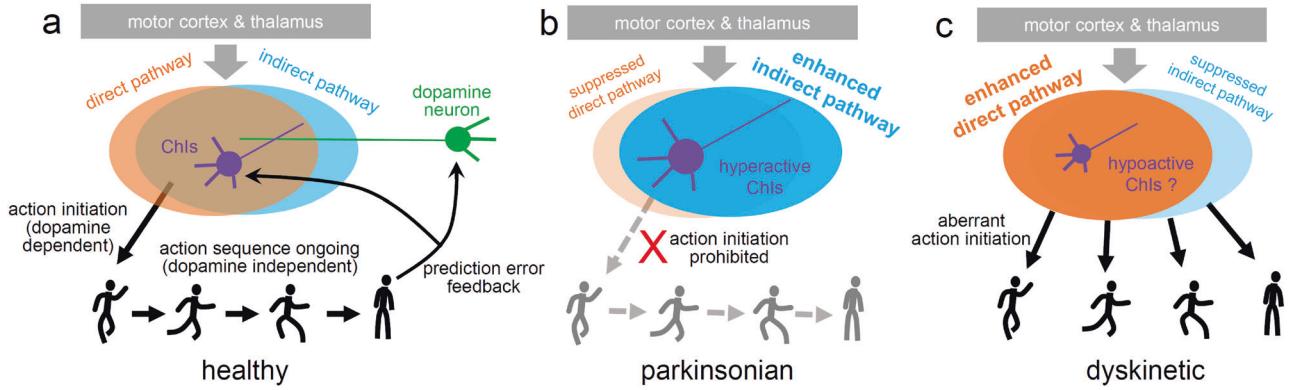

Fig. 1 Diagrams of the striatal motor control system in health and pathology. **a** Schematic showing the organizing principle of the motor control system in the brain. The activities of the direct and indirect pathways in the striatum are indicated by orange and blue ovals, respectively. Dopamine neurons (green) from the midbrain and striatal ChIs (purple) are also shown. In the healthy brain, action sequences are encoded in the cortex and thalamus, transferred to the striatum (gray arrow), and initiated immediately after a brief dopamine transient and acetylcholine release. Once the movement kicks off, the actions (movement icons) are sequentially performed in a dopamine-independent manner. A highly coordinated interplay of striatal circuitry governs the execution of action sequences, with the direct pathway (orange) facilitating the performance of the appropriate actions and the indirect pathway (blue) suppressing unwanted ones. The precise balance of activity between the two pathways is essential for the accurate performance of motion sequences (indicated by the merged area with similar brightness of each color). Once the movement is finished, the consequence of the motion is evaluated, and a feedback signal of prediction error is generated in both ChIs and dopamine neurons for Hebbian modification of the striatal circuitry. If the circuits involved in the motion generate positive consequences for survival, they are enhanced (through the formation of synaptic LTP) to make them easier to recruit in the future. In the opposite scenario, if the behavioral consequences are worse than expected, the responsible circuit will be undermined (through the formation of synaptic LTD) and will be harder to activate thereafter. This functional feedback loop underlies the basis of motor learning in the striatum, where ChIs and dopamine neurons play essential roles in both the action initiation and result evaluation phases. **b** In parkinsonian conditions, dopamine neurons are lost. Falling dopamine levels in the striatum generate aberrant homeostatic adaptations in striatal neurons and synaptic plasticity in the striatal circuitry. ChIs become hyperactive and fire more synchronously. MSNs undergo homeostatic changes trying to restore the balance over time. The intrinsic excitability of MSNs of the direct pathway increased due to long-term loss of D1 activation, and the excitability of MSNs of the indirect pathway decreased due to loss of D2 activation. The bidirectional synaptic plasticity at cortical striatal synapses is the key cellular basis for motor learning and movement control. Nevertheless, since there is not enough dopamine left in PD, no LTP can form in the direct pathway while no LTD can form in the indirect pathway; this aberrantly suppresses the direct pathway (illustrated as the lighter orange oval) but artificially reinforces the indirect pathway (illustrated as the darker blue oval). Hence, movement commands prefer to flow through the indirect pathway but not through the direct pathway, generating an enhanced "stop" signal and a diminished "go" signal (dashed arrows). Without dopamine, feedback on behavioral consequences is not generated, and no proper motor learning occurs in the striatum. **c** When PD patients are treated with levodopa, the striatal circuitry is constantly bombarded by abnormally sustained dopamine stimulation. Although levodopa administration can restore LTP and LTD formation in striatal synapses, it fails to replicate the spatiotemporal pattern of dopamine signaling in the healthy brain. As a result, synaptic strength is no longer governed by the outcomes of behaviors but is erratically regulated. Since higher dopamine levels prefer to strengthen the direct pathway (illustrated as the darker orange oval) but suppress the indirect pathway (illustrated as the lighter blue oval), unwanted actions are not sufficiently suppressed by the indirect pathway, causing random execution of movement (arrows and movement icons). Reduced ChI activity and cholinergic transmission have been reported after long-term levodopa treatment but contradicting evidence exists suggesting that ChIs might still be hyperactive

Using in vivo calcium imaging, they characterized the activity of the direct and indirect pathways in the striatum of freely moving animals and found that both pathways were concurrently activated during action initiation and execution, opposing the long-held prediction of the classical model that the direct pathway is specifically involved in movement initiation and that the indirect pathway is solely responsible for terminating the ongoing action [21, 28, 29]. Additionally, their research found that excitation or inhibition of either pathway delayed the initiation of movement and impaired the continuity of a learned movement sequence. Interestingly, the performance of an action sequence can be fine-tuned by subtle activation of the direct pathway and aborted by activation of the indirect pathway [30]. Together, these data indicate that both the direct and indirect pathways are necessary for action sequence execution, with the direct pathway facilitating the performance of a running action and the indirect pathway permitting it by inhibiting other competing actions (Fig. 1a). Hence, action selection and movement initiation in the striatum is most likely mediated by a highly regulated dynamic balance between the two complementary pathways in the striatum. It is the specific activity patterns, rather than activity levels, that are critical for appropriate action initiation and selection [22, 30].

RETHINKING THE ROLE OF DOPAMINE IN MOTOR CONTROL

Although the importance of dopamine in movement regulation is widely recognized, it is not entirely clear how dopamine regulates the inputs and outputs of the striatum. The classical model states that since D1 and D2 receptors oppose each other in cyclic AMP production, activation of D1 receptors enhances the activity of the direct pathway, whereas activation of D2 receptors exerts opposite impacts on the indirect pathway. As a consequence, an increase in dopamine levels would shift the balance to favor the control of the direct pathway [13, 22, 31]. This working principle is essentially still a rate model that relies on dopamine levels to generate "go" and "stop" signals. Several recent studies, however, have indicated that this model seems to be too rigid to account for the complexity of dopamine regulation. On the one hand, both pathways enhance their firing activity simultaneously to initiate actions when dopamine is released, which is inconsistent with the prediction of the classical model that dopamine increases direct pathway activity and decreases indirect pathway activity [28, 29]. On the other, in vivo studies have indicated that activation of either D1 or D2 receptors can bidirectionally regulate the excitability of both pathways, in apparent contrast with the assumption that dopamine excites the direct pathway but inhibits the indirect pathway [14].

Thanks to the development of optogenetic indicators, dopamine dynamics concerning motor control were recently uncovered. Using in vivo calcium imaging of dopamine innervation to the striatum, the Costa laboratory and Dombeck laboratory have independently demonstrated that time-locked burst firing in dopamine axons is causally required for action execution, suggesting that it is the temporal dynamic of dopamine, not merely the dopamine level, that is responsible for the control of movement [31–34]. Another important finding of their studies is that once the movement is initiated, dopamine is dispensable for subsequent actions, opposing the classical model that dopamine levels constantly regulate striatal output [35, 36]. It has been postulated that motion sequences are performed either in a serial manner, in which the end of one element triggers the start of another, or are represented and controlled in a hierarchical manner, in which the number and order of elements are preprocessed before the commencement of movement [37, 38]. The fact that dopamine is required for the initiation phase but not for the ongoing phase of the movement sequence suggests that action sequences are probably represented hierarchically in broad neural networks, and dopamine transients most likely provide a brief gating signal in a precisely timed manner to invigorate the preplanned serial movements (Fig. 1a) [14, 30, 38].

Despite generating immediate impacts on the activity of striatal circuits, dopamine also governs motor learning by regulating the strength of synaptic connections originating from afferent inputs [39]. Motor learning is a simultaneous decision-making process of what to do by the direct pathway and what not to do by the indirect pathway. When the consequence of a particular behavior turns out to be positive, a brief dopamine transient is produced to enforce the strength of the recruited circuits, making them more likely to be activated in the future. Instead, if the consequence has an adverse outcome, a temporary drop in dopamine level prohibits the strengthening of the engaged circuit. Consistent with this working model, under normal conditions, dopamine transients indeed dominate the formation of spike-timing-dependent long-term potentiation (LTP) in the direct pathway and long-term depression (LTD) in the indirect pathway [31]. One caveat of this model is that the activity of dopamine axons originating from the substantia nigra does not change in response to behavioral consequences. It is thus most likely that dopamine projections from the ventral tegmental area (although relatively few) carry the feedback information [35, 40]. The long-term modulation of striatal circuitry by dopamine is essentially Hebbian learning, which highly relies on the precise timing of dopamine signaling, and the bidirectional nature of this regulation enables full-fledged motor control of the striatal circuitry (Fig. 1a) [41, 42].

While dopamine signaling has long been presumed to occur through volume transmission, which is considered slow and inaccurate, new lines of evidence have indicated that there may be more to consider [43, 44]. First, some dopamine terminals can form synapse-like structures through neuroligin-2, a cell adhesion protein found on the postsynaptic membrane that mediates synapse formation [45]. Second, dopamine release highly relies on very specialized release sites along their axons, indicating a very organized architecture in the striatum [46–49]. Third, functional studies have demonstrated that dopamine release is exceptionally fast and efficient, and the release ability quickly declines following the initial transient, which fits well with the functional requirement of initiating movement but not supporting ongoing movement [47, 50, 51]. Finally, in addition to activating dopamine receptors, dopamine terminals can co-release GABA, generating fast inhibition in both pathways [52–54]. Together, these properties suggest that dopamine signaling is much more spatiotemporally controlled than previously thought, supporting the functional obligation for time-locked tuning of the striatal activity and synaptic plasticity.

STRIATAL CHOLINERGIC SYSTEM

The striatum has the highest level of acetylcholine in the brain, most originates from local ChIs, with a small amount coming from the brainstem [55–57]. ChIs are huge cells (30–50 μm in diameter), possess extremely arborized axons, and make widespread connections throughout the striatum. It was estimated that, on average, each ChI can generate as many as half a million terminals [58–60]. ChIs mainly make two types of connections within the striatum. One is axodendritic synapses with distal dendrites and dendritic spine necks of MSNs, and the other is axoaxonal connections with afferent glutamatergic and dopaminergic terminals [57, 61–65]. In addition to exerting direct influences on synaptic sites, cholinergic terminals have also been suggested to be able to act through volume transmission, which can generate widespread cumulative impacts on nearby neurons [66].

ChIs display unique electrophysiological properties. They exhibit high input resistance, broad action potentials, and a pacemaking firing of 2–10 Hz [67]. The depolarization phase of the spontaneous firing is controlled by hyperpolarization and cyclic nucleotide-activated cation (HCN) channels, and the repolarization phase is governed by calcium-activated potassium channels [68–70]. The external regulation of ChIs was traditionally considered to arise mainly from the thalamus [61, 71], but recent studies using monosynaptic rabies virus tracing have argued that cortical glutamatergic innervation is most likely the primary afferent input of ChIs [72, 73]. Notably, more than half of the synapses on ChIs arise from local MSNs and GABAergic interneurons, indicating that ChIs also receive significant inhibitory regulation from within the striatum [73–75]. Another essential extrinsic regulatory mechanism of ChI activity is its modulation by dopamine. Almost all ChIs express dopamine D2 and D5 receptors, and a small fraction of ChIs (~20%) also express D1 receptors [76, 77]. As D2 and D5 receptors are coupled with functionally opposing G proteins (Gi and Gs, respectively), dopamine can thus bidirectionally regulate cyclic AMP levels and their corresponding actuators within ChIs [78]. Activation of D2 receptors slows down the firing rate and acetylcholine release [77, 79–81]. Activation of D5 receptors has been shown to play a crucial role in the formation of LTP at synapses on ChIs [82–84].

There are two types of cholinergic receptors in the striatum: muscarinic receptors (mAChRs) and nicotinic receptors (nAChRs) [85]. Overall, mAChRs significantly outnumber nAChRs in the striatum. mAChRs are G-protein coupled receptors that can be subdivided into two classes: Gq-coupled (M1, M3, and M5) and Gi-coupled (M2 and M4). The M4 subtype is the most abundant mAChR in the striatum. Activation of Gq-coupled mAChRs generally enhances synaptic transmission, increases the excitability of neurons, and facilitates the formation of LTP, while activation of Gi-coupled receptors does the opposite. nAChRs are pentameric ion channels and permeable to both sodium and calcium ions when opened. There are also two subclasses of nAChRs: nAChRs composed of αβ subunit combinations and homomeric nAChRs made up of only α subunits. The most abundant type of nAChR formed in the striatum is the α4β2 nAChR. Although ChIs express all types of mAChRs and low levels of α7-containing nAChRs, M4 autoreceptors dominate the regulation of ChI activity in response to acetylcholine release. Activation of M4 receptors reduces firing activity, calcium influx, and acetylcholine release, providing robust feedback inhibition for ChIs (Fig. 2). This property significantly differs from that of other cholinergic neurons projecting to the hippocampus and cortex, in which autoinhibition is mediated through M2 receptors [85–89].

CHOLINERGIC REGULATION OF STRIATAL CIRCUITRY

Although low in number, ChIs integrate a multitude of inputs and exert significant influences on striatal output. In contrast to dopaminergic regulation, cholinergic modulation does not seem

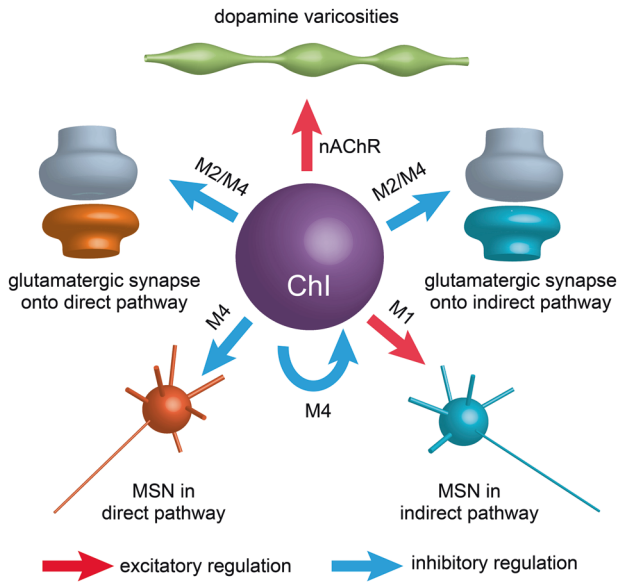

Fig. 2 Schematic illustration of cholinergic regulation in the striatum. ChIs exert influences on striatal function by regulating multiple targets (arrows). Activation of ChIs can reduce glutamatergic transmission to MSNs of both pathways via M2 and M4 mAChRs, trigger dopamine release from their terminals through nAChRs and generate feedback inhibition via M4 receptors. Although MSNs of both the direct and indirect pathways express M1 receptors, which increase the excitability of a neuron when activated, the direct pathway is inhibited by acetylcholine because of the high expression level of M4 receptors

to strictly distinguish between the direct and indirect pathways. Optogenetic activation of ChIs inhibits ~80% of MSNs from both pathways and excites the rest [14]. There are three major pathways through which the striatal cholinergic system regulates the activity of the MSNs: glutamatergic innervation of MSNs, intrinsic excitability of MSNs, and striatal dopamine release. The net effect of the cholinergic modulation of MSN activity is thus determined by the complex interactions between these pathways, which, although hard to interpret, enables fine adjustment of the striatal circuitry at multiple levels (Fig. 2).

It has long been known that acetylcholine modulates striatal glutamate release from the presynaptic terminals of MSNs. Local application of acetylcholine or increasing the firing of ChIs significantly reduces excitatory transmission onto MSNs through the activation of mAChRs [90–93]. While both M2 and M4 mAChRs are found in the glutamatergic afferent terminals and both receptors activate Gi/o proteins, M4 receptors are thought to be primarily responsible for this inhibition [93, 94]. Given that ChIs have spontaneous activity, glutamatergic terminals are likely under tonic inhibition by acetylcholine [91]. Since the majority of glutamatergic terminals originate from the cortex and thalamus, the regulation is considered to function as a filter of external movement control commands [73, 92, 95]. In addition to mAChRs, there is also a significant number of nAChRs that reside in glutamatergic terminals [96–98]. As nAChRs are permeable to calcium, the opening of these channels should, in theory, increases the release probability of glutamate. Consistent with this hypothesis, stimulating nAChRs increases glutamate levels in the striatum, as measured using microdialysis [98]. In addition, it was recently shown that a subset of afferent glutamatergic synapses from the thalamus is specifically enhanced through upregulated activation of nAChRs in a mouse PD model [99]. It appears that the regulation of glutamatergic terminals by ChIs is dichotomous, but it is unclear why the overall influence of broad acetylcholine stimulation is always the suppression of glutamate release? One possibility is that the impact of mAChR activation lasts much longer than that of nAChR activation, the effects of which decline exceptionally fast due to receptor desensitization [90–93]. It is also possible that nAChRs and mAChRs are expressed in different terminal subsets and that the appearance that mAChR activation dominates the effect of acetylcholine is simply because mAChR-expressing terminals outnumber nAChR-expressing terminals [73].

MSNs express mAChRs but are devoid of nAChRs, and the expression patterns are considerably different between the direct and indirect pathways. While MSNs in both pathways express M1 receptors, MSNs in the direct pathway also express a significant number of M4 receptors [94, 100]. Activation of M1 receptors stimulates Gq proteins and leads to corresponding changes in a multitude of ion channels, including Kv potassium channels, Kir2 channels, Nav1 sodium channels, and Cav2 calcium channels [101–103]. The net outcome of these regulations is generally an increase in dendritic excitability [104]. Notably, activation of M1 receptors increases the excitability of the indirect pathway much more strongly than it increases the excitability of the direct pathway, likely due to different expression levels of the receptor or its corresponding actuators [105]. In contrast, activation of M4 receptors tends to attenuate dendritic excitability, promote the formation of LTD, and suppress the formation of LTP in the projections of the direct pathway [106]. Given that only MSNs in the direct pathway express M4 receptors, the overall effect of acetylcholine is to attenuate the intrinsic excitability of the direct pathway while promoting that of the indirect pathway, opposing the influences of dopamine.

The most exciting function of ChIs in the striatum is their regulation of dopamine release (Fig. 2). Dopamine neurons express high levels of nAChRs composed of α4, α6, and β2 subunits. More than half of nAChRs in the striatum reside in dopamine terminals [64, 107–109]. Early pharmacological studies suggested that activation of nAChRs promotes dopamine release [62, 110, 111]. Later, using voltammetric recordings in brain slices, it was revealed that the motivation of endogenous ChIs induces much more dopamine release than activation of dopamine axons alone [57, 112–114]. An important recent finding is that synchronous activation of ChIs can trigger dopamine release from dopamine terminals directly, independent of activity from dopamine cell bodies [61, 115, 116]. As dopamine neurons co-release GABA, activation of ChIs also triggers significant among of GABAergic currents in MSNs through dopamine axons [117]. The mechanism of the cell body-independent release is still unclear. Since nAChRs are permeable to calcium, this release may be induced directly by the calcium influx through these channels. Nevertheless, it is also possible that the depolarization caused by nAChRs will induce an ectopic action potential that not only triggers dopamine and GABA release locally but broadcasts these signals along the extremely arborized dopamine axonal network [40]. The finding that activation of striatal ChIs induces dopamine release blurred the boundaries between acetylcholine and dopamine in the striatum. It is entirely possible that many effects of the cholinergic modulation on striatal circuitry are actually exerted through dopamine. Further research is required to investigate when and how this mechanism is employed in vivo.

ROLE OF CHIS IN HEALTH

Striatal ChIs serve as information processing nodes by receiving inputs from a variety of neurons and integrating them to influence behavior through multiple regulatory pathways. The firing activity of ChIs in vivo varies depending on the behavioral context. When burst firing of ChIs is induced by glutamatergic innervation from the cortex and thalamus, the corresponding acetylcholine transient immediately suppresses the flow of information into MSNs by activating presynaptic M2/M4 receptors on glutamatergic terminals

and increases the intrinsic excitability of the indirect pathway via M1 receptors (Fig. 2) [92]. In freely moving animals, ChIs exhibit rapid and transient firing across the whole population before the onset of spontaneous locomotion, and the synchrony of ChI firing diminishes as animals transit to continuous movement, a pattern very reminiscent of that of dopamine neurons [36, 118]. Simultaneous recordings of ChIs and dopamine neurons, however, indicate that the activities of the two populations are coordinated but not correlated, suggesting that they code distinct aspects of the movement. No significant change or a slight reduction in the amount of locomotion is observed when ChIs are directly recruited using light, but unilateral ChI ablation can cause turning behavior [119–121]. Multiple lines of evidence indicate that ChIs are most likely responsible for the expression of behavioral flexibility to changed surroundings [122–125]. Most studies support a positive correlation between the activity of ChIs and motor shift [126–128], but one report indicated that the removal of ChIs enhances motor flexibility [129].

Another featured activity of ChIs in vivo is the pause-rebound firing pattern in response to motivationally significant events, in which ChIs reduce the firing rate when the sensory cue is presented and increase it immediately afterward. This response is acquired after reward-paired training and has been suggested to play a prominent role in motor learning. The acquisition of the response depends on the dopaminergic system, but ChIs do not simply reflect the firing activities of dopamine neurons [19, 20, 82, 130]. In contrast, the pause coincides with increased firing of dopamine neurons [130, 131]. Given that ChIs tonically inhibit glutamate release, the reduced activity of ChIs during cue presentation might permit the flow of more information into the striatum, consolidating striatal circuits synergistically with dopamine [130, 131]. The mechanisms of the pause-rebound activity are still under debate. Some believe it is solely caused by dopamine regulation [132, 133]. Others argue that both synaptic and intrinsic mechanisms can induce it in the absence of dopamine modulation [134, 135]. This discrepancy might arise from the heterogeneity of ChI distribution and regulation. Studies have shown that dopamine can inhibit ChIs residing in the dorsomedial striatum but excite ChIs in the dorsolateral area [136].

ROLE OF CHIS IN PD

A range of adaptations occurs in the striatum with the progression of PD [137–140]. The prevailing theory of the motor symptoms of PD is that the loss of dopamine in the striatum causes an imbalance between the direct and indirect pathways, with the direct pathway suppressed and the indirect pathway overexcited. Considering that the two pathways compete with each other in movement selection, these pathological alterations might shift the equilibrium between the two to favor blocking the proper relay of movement control commands, causing the hypokinetic symptoms of PD (Fig. 1b) [21, 141]. Although ChIs also highly express several PD causal genes (i.e., LRRK2), as do dopamine neurons, the accumulation of α-synuclein and the loss of ChIs are only observed in late PD [138, 142]. Associated with PD progress, both the acetylcholine level and activity of ChIs are highly elevated [106, 143]. Since ChIs tend to weaken the direct pathway and promote the indirect pathway, the elevated ChI activity is believed to exacerbate the PD symptoms (Fig. 1b) [99, 141]. Surprisingly, the elevated ChI activity is not caused by a loss of D2 inhibition as one would intuitively expect but is attributed to the attenuation of M4 autoreceptors, indicating that upregulation of acetylcholine signaling is not a byproduct of dopamine depletion but likely an active driver of striatal adaptations [89, 106]. Although the lack of D2 activation does not contribute much to the altered excitability of ChIs, it does reduce the pause duration of ChIs after burst firing. In addition to altered excitability, the firing of ChIs also becomes much more synchronized in PD models, likely due to elevated afferent inputs [92, 99, 144]. As synchronous activation of ChIs can trigger dopamine release directly, the increase in synchronicity might serve as a compensatory mechanism for dopamine reduction at the early stage of PD.

No significant changes have been found in the overall expression level of M-type receptors in mouse PD models, but whether there are differences in the level of regulation among subtypes has also not been fully determined [145]. On the other hand, nAChRs are gradually lost following dopamine depletion in both animal models and clinical cases [85, 146]. Among nAChRs, the α4- and α6-containing subtypes are usually the first to be lost, most likely because these receptors mainly reside in dopamine axons [147].

NACHRS AS A TARGET FOR PD TREATMENT

There are currently no disease-modifying drugs or approaches for treating PD, and most therapies focus only on managing PD symptoms [148]. The primary beneficial effects of these treatments are to help manage PD symptoms, to alleviate levodopa-induced dyskinesia (LID), and to improve the cognitive impairments associated with the disease. The predominant view postulates that dopamine and acetylcholine play opposing roles in motor control and that the balance shifts towards acetylcholine in PD [89, 140, 149]. Consistent with this view, optogenetic inhibition of ChI activity indeed alleviates PD symptoms in several animal models of PD [120]. Although mAChR antagonists have long been shown to effectively reverse PD motor symptoms, the cognitive and autonomic side effects have prevented them from being widely used, and their use quickly waned after the introduction of dopamine replacement therapy with levodopa [150]. Today, levodopa is still the gold standard for PD treatment. However, levodopa has a short therapeutic window, and prolonged levodopa administration generates several side effects, including mood disturbances and dyskinesia; thus, there is a critical need to improve treatments for PD [151–153].

The new findings regarding the cholinergic system discussed above unravel several potential therapeutic targets in the striatal cholinergic system and support a re-emergence of cholinergic treatment for PD. Several lines of evidence indicate that nAChRs might serve as a promising drug target for those purposes [97, 99, 154, 155]. First, there is an extensive anatomical and functional overlap between nAChRs and dopamine projections within the striatum. Activation of nAChRs in dopamine axons can modulate and even directly trigger dopamine release. Second, various expressional and functional adaptations of nAChRs occur in association with PD progression and contribute to the expression of PD symptoms [99]. Third, drugs that interact with nAChRs might protect against dopamine neuron degeneration [146, 156, 157]. The potential ability of nAChRs to rebalance the direct and indirect pathways is compatible with the therapeutic requirements for treating PD-related movement disorders [97, 99, 154, 155].

Immediate questions are which nAChR subtype should be targeted and whether the nAChR function should be up- or down-regulated. Given that nAChRs are significantly impaired in PD, it appears that restoring the function of nAChRs should be the direction to go. Since activation of nAChRs can trigger dopamine release, stimulating nAChRs might boost striatal dopamine levels, compensating for dopamine deficiency. Nevertheless, the treatment strategy is likely highly dependent on the stage of the disease. At late-stage PD, since there are much fewer dopamine terminals left in the striatum, nAChR stimulation might have a very limited impact on dopamine signaling but preferentially regulate the transmission of other neuronal innervations. Consistent with this idea, a recent report indicated that thalamostriatal projections to the indirect pathway are specifically enhanced by the overactivation of nAChRs in a late-stage PD mouse model.

Importantly, inhibiting nAChRs, but not activating them, helps with motor deficits [99]. The paradox that both activation and inhibition of nAChRs can be beneficial to PD might also arise from the fast desensitization properties of the receptors themselves. It has been shown that even the amount of nicotine administered by smoking efficiently desensitizes nAChRs, making it possible that long-term stimulation of nAChRs functionally inhibits, rather than activates, nAChR signaling in the striatum [65].

Another potential therapeutic strategy for manipulating the striatal cholinergic system is alleviating LID. A number of therapeutic strategies, including delaying the onset of levodopa treatment and reducing the levodopa dose, have been employed to minimize the onset of LID. However, these approaches inevitably compromise the control of PD symptoms [158, 159]. Strategies to treat LID are currently very limited, and there is a tremendous unmet need to identify new therapies. Several lines of evidence indicate that aberrant LTP formation and hypersensitivity of the direct pathway, together with strong inhibition of the indirect pathway, underlie this pathology (Fig. 1c) [160, 161]. These conditions are likely caused by the sustained activation of dopamine receptors during levodopa therapy. The strength of afferent inputs of the direct pathway are typically balanced by both dopamine and acetylcholine. Activation of dopamine D1 receptors enhances the formation of LTP, and activation of cholinergic M4 receptors (which are only expressed in the direct pathway) facilitates the formation of LTD [106, 162, 163]. Although it appears that M4 signaling should be enhanced to counterbalance the influence of constant D1 activation during levodopa treatment, activation of mAChRs through optogenetic stimulation of ChIs worsens this condition [153]. In contrast, the ablation of ChIs or activation of nAChRs results in significant improvements in symptoms [152, 153, 164, 165]. The effectiveness of the approach seems to be dependent on the stage of PD, and none of the treatments reduces the action of levodopa, indicating that the mechanism is distinct from modulations linked to PD [166]. Consistent with the idea that nAChRs play central roles in the treatment, both the development of dyskinesia and the therapeutic benefit of nicotine are reduced in nAChR knockout mice [154, 167].

The progress of PD is also accompanied by several nonmotor symptoms, including sleep disorder, depression, and cognitive impairment that eventually develops into dementia [168]. The pathology of cognitive impairment is complex and involves the degeneration of several systems, a condition very reminiscent of Alzheimer's disease. Regarding the cholinergic system, significant cholinergic neuronal loss and decreases in several subtypes of nAChRs have been found to be associated with the progression of cognitive decline [169, 170]. Acetylcholinesterase inhibitors, which are commonly used to treat Alzheimer's disease, are very effective in boosting cognition in PD patients [171]. Unfortunately, increasing the level of acetylcholine in the brain exacerbates motor deficits in PD and is thus not an ideal approach. On the other hand, since acetylcholine levels are positively correlated with cognition, treating PD with anticholinergic drugs deteriorates cognition [172]. Intense research has focused on α7 nAChR ligands, and some studies have reported that these drugs have positive effects on cognition [173, 174].

NICOTINE AS A DRUG CANDIDATE FOR PD TREATMENT

Although the complexity of PD makes it extremely difficult to predict whether or which nAChRs can generate a beneficial effect, the use of nAChR agonists for the treatment of PD has been studied for over 3 decades. Drugs targeting β2-containing nAChRs have been shown to ameliorate PD symptoms in several animal PD models [175]. Compounds that stimulate α7-containing nAChRs have been reported to slow down the degeneration of dopamine neurons [176]. Nonetheless, the star candidate is an old compound: nicotine. Nicotine is a plant alkaloid present in tobacco and a nonselective nAChR agonist. It exhibits the highest binding affinity ($K_d < 1$ nM) for the α4β2 nAChRs and lowest binding affinity for α7-containing nAChRs ($K_d > 1$ μM) [177]. Several clinical trials have claimed that nicotine reduces motor symptoms in PD patients, but others found it ineffective [155, 178–180]. These discrepancies most likely arise from the design of the studies (many of them did not include a placebo control group) and the different severity of the patients recruited.

Epidemiological studies have consistently shown that smoking is inversely related to susceptibility to PD [157]. While tobacco contains thousands of components, nicotine stands out due to its relatively high abundance in tobacco and its interactions with nAChRs. If its potential neuroprotective effect is real, nicotine or nAChRs will represent a new milestone for PD treatment since current therapies only address the symptoms of PD. Preclinical studies have indeed provided some hints of this possibility. First, nicotine can prevent aggregation of both wild-type and A53T mutant α-synuclein in tubes [181]. Second, in several cell culture models of PD, nicotine treatment can significantly decrease cell loss [182, 183]. Finally, in both 6-OHDA-induced rodent and MPTP-induced primate models of PD, nicotine administration can slow down dopamine neuron degeneration [184, 185]. The mechanisms of the possible neuroprotective effect are still enigmatic. Some biochemical studies have indicated that nicotine might upregulate anti-apoptotic proteins to slow down cell death and enhance the expression of enzymes of the P450 family to reduce neurotoxins [186, 187]. Others using single-cell transcriptomics of midbrain dopamine neurons have identified several genes regulated by nicotine treatment. Interestingly, nicotine did not influence nAChR genes but regulated a series of genes that might contribute to its neuroprotective effect, including genes associated with the ubiquitin-proteasome pathway, cell cycle regulation, and chromatin modification [188]. It is essential to point out that nicotine only protects against ongoing degeneration rather than restore damaged neurons, suggesting that nicotine-based treatments would only be valid in the early stages of PD [189].

Although some clinical trials have reported improvements in PD symptoms after nicotine treatment, these early studies were generally performed over an observation period of several weeks, which is too short to test any disease-modifying potentials of nicotine. A recently completed placebo-controlled and double-blind multicenter trial, however, reported that chronic transdermal application of nicotine does not influence the progression of PD [179, 180]. One possible explanation for the failure of this trial is the U-shaped dose-response curve of nicotine. Maximal protection is only reached with an intermediate dose, but the clinical trial used a high dose [179, 190]. It is also possible that the benefit of smoking arises from the synergistic effect of other components in tobacco, as smoking also reduces monoamine oxidase activity in the brain, which contributes to the protection of dopamine neurons [191].

On the other hand, long-term nicotine treatment consistently suppresses LID without developing significant tolerance in several PD animal models [166, 192]. It seems that the effectiveness of nicotine depends on the formation of synaptic plasticity because the therapeutic benefits require chronic administration of the drug and because the effects of the drug are maintained for several weeks after treatment cessation [193]. Nevertheless, the efficacy of nicotine is partially determined by the disease stage and the integrity of nAChRs. Nicotine is only effective in dealing with mild or moderate parkinsonian states but not in treating severe conditions when dopamine neurons are completely lost, likely due to the relatively low number of nAChRs that remained in the late stage of PD [191].

OUTLOOK

Substantial efforts toward discovering new therapeutic approaches for the management of PD have been made. Recent progress on

the functional dissection of the striatal network has reshaped our understanding of the physiology and pathology of motor control and has shed light on new directions for treating PD symptoms. Although several new reciprocal interactions between the cholinergic and dopaminergic systems have recently been identified, the general principle that the two transmitters functionally antagonize each other in the process of motor control still holds. Suppressing ChI signaling seems to be beneficial for both controlling PD symptoms and slowing down the expression of LID. Despite the complex interactions and adaptations that occur in the striatum in PD, nAChRs are emerging as a promising drug target due to safety and effectiveness compared to other candidates. Although several approaches have been demonstrated to be very effective in rebalancing the direct and indirect pathways, it is essential to point out that none of them alleviated the motor learning deficits associated with severe dopamine decline. Restoring dopaminergic innervation or signaling pathways will be the ultimate goal for PD therapy, which relies on a much deeper understanding of motor control and a combination of various therapeutic strategies beyond pharmacological approaches.

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ADDITIONAL INFORMATION

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