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Review Article

The muscarinic M_4 acetylcholine receptor exacerbates symptoms of movement disorders

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Barbeau's seesaw hypothesis of dopamine-acetylcholine balance has predominated movement disorders literature for years. Both the simplicity of the explanation and the matching efficacy of anticholinergic treatment in movement disorders seem to support this hypothesis. However, evidence from translational and clinical studies in movement disorders indicates that many features of this simple balance are lost, broken, or absent from movement disorders models or in imaging studies of patients with these disorders. This review reappraises the dopamine-acetylcholine balance hypothesis in light of recent evidence and describes how the $G\alpha_{i/o}$ coupled muscarinic M_4 receptor acts in opposition to dopamine signaling in the basal ganglia. We highlight how M_4 signaling can ameliorate or exacerbate movement disorders symptoms and physiological correlates of these symptoms in specific disease states. Furthermore, we propose future directions for investigation of this mechanisms to fully understand the potential efficacy of M_4 targeting therapeutics in movement disorders. Overall, initial evidence suggest that M_4 is a promising pharmaceutical target to ameliorate motor symptoms of hypo- and hyper-dopaminergic disorders.

Introduction

Canonical literature in movement disorders and basal ganglia physiology suggests a fine-tuned balance between levels of the neurotransmitters dopamine (DA) and acetylcholine (ACh; Barbeau's seesaw hypothesis; [1,2]). This viewpoint has been largely supported by observed changes in cholinergic transmission in disorders affecting DA such as Parkinson's disease (PD) and schizophrenia [3,4]. Additional clinical evidence points to this imbalance: hypo-DAergic movement disorders can be treated with anticholinergic therapies, whereas hyper-DAergic disorders can be treated with pro-cholinergic therapies [5,6]. The seesaw view of ACh and DA is an oversimplification which largely hinges on research describing levels of neurotransmitters. However, neurotransmitter levels alone do not directly indicate or correlate to neural activity or plasticity in the basal ganglia circuit. Furthermore, the relationship between DA and ACh is more nuanced than simply two neurotransmitters suppressing one another, as there are complex signaling mechanisms among a number or receptors that bind these neurotransmitters. DA acts in opposition to acetylcholine release in the striatum, via $G\alpha_{i/o}$-coupled DA D2 receptors (D_2) expressed on striatal cholinergic interneurons (ChI; [7,8]). However, there is considerable overlap in expression of DA receptors and ACh receptors in spiny projection neurons (SPNs), corticostriatal terminals, and even in ChI, which suggests a more complicated interplay between ACh and DA receptor signaling. Conversely, ACh exerts bidirectional effects on DA release. Specifically, ACh can both increase nigrostriatal DA release through activation of nicotinic receptors [9] and can also both oppose or facilitate the effects of DA release and signaling depending on which muscarinic ACh receptors it activates on spiny projection neurons (SPNs; [10,11]),corticostriatal glutamate terminals [12], or DA terminals themselves. In particular, recent research has

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highlighted a unique role for the M_4 muscarinic ACh receptor (M_4) specifically in regulating DA signaling and direct pathway plasticity.

M_4 are metabotropic ACh receptors which are coupled to $G\alpha_{i/o}$. ACh binding to M_4 leads to the inhibition of adenylyl cyclase and subsequently decreases cyclic adenosine monophosphate (cAMP). Of note, M_2 muscarinic ACh receptors show significant functional overlap with M_4; however, unlike M_4, their location in peripheral heart and lung tissue precludes them from being a safe and effective therapeutic target for central nervous system disorders [13]. M_4 are most abundantly expressed in the striatum, and mechanistic electrophysiological and behavioral studies using newly developed pharmacological tool compounds have indicated several key sites of M_4 regulation of the basal ganglia motor circuit (See Figure 1). First, at corticostriatal terminals, M_4 inhibits glutamate release [12]. Second, M_4 is thought to inhibit ACh release from striatal ChIs [14,15]. Third, M_4 acts on cell bodies and terminals of direct pathway spiny projection neurons (dSPNs; [10,11,16]), where they directly oppose $G\alpha_{olf}$-coupled DA D1 receptor (D_1) signaling and promote dSPN long-term depression (LTD). When ACh binds to M_4 on dSPN cell bodies, it also reduces the probability of DA release through autacoid

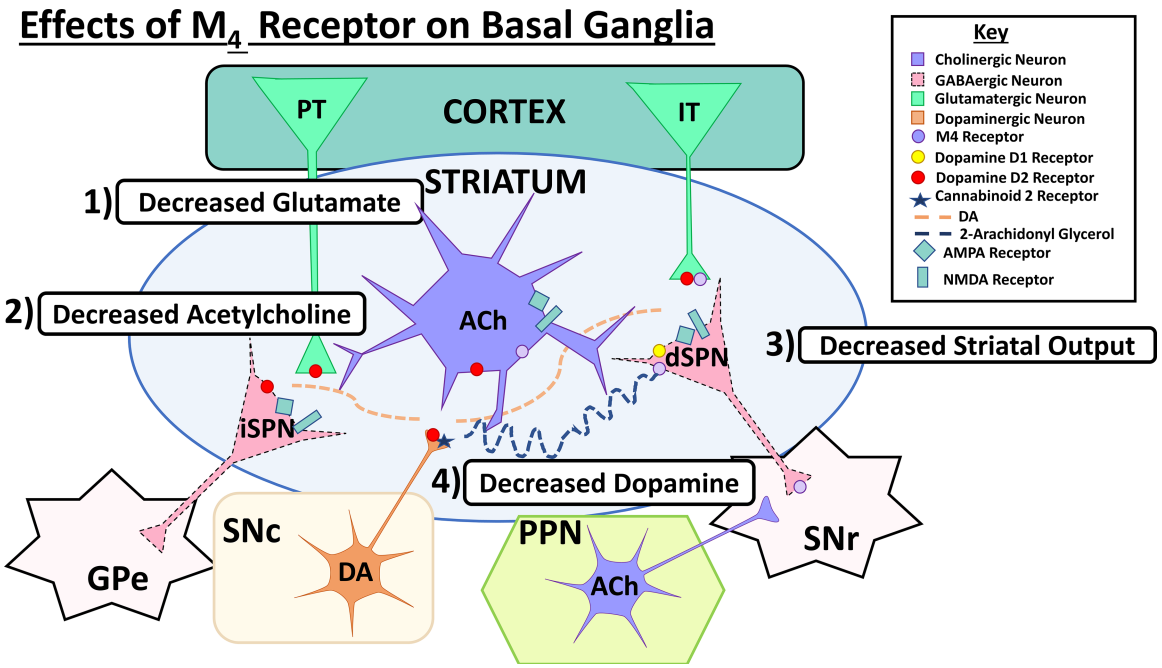

Figure 1. Pathways for M_4 modulation of movement.

$G\alpha_{i/o}$-coupled M_4 muscarinic acetylcholine (ACh) receptors exert four main effects on basal ganglia signaling through their expression on several key neurons, namely corticostriatal glutamate terminals, striatal cholinergic interneurons, and direct pathway spiny project neurons (dSPN). The most important effects of M_4 signaling are summarized in this figure. (1) M_4 are located on terminals of corticostriatal glutamate projections (mainly on intratelencephalic neurons (IT) and less so on pyramidal tract neurons(PT)), where they provide long-term inhibition of glutamate release, thus limiting excitation of striatal spiny projection neurons and striatal long-term potentiation. (Please note that IT and PT neurons synapse onto both direct pathway spiny projection neurons (dSPN) and indirect pathway spiny projection neurons (iSPN)). (2) M_4 are present on striatal cholinergic interneurons, where they serve as an autoreceptors and decrease probability of ACh release. (3) Most importantly, M_4 counter the effects of DA signaling through their expression on cell bodies and terminals of dSPN. M_4 located on dSPN terminals in the substantia nigra pars reticulata (SNr) provide an axonal target for direct pathway inhibition when they receive brainstem ACh from the pedunculopontine nucleus (PPN). (4) Interestingly, ACh binding to M_4 located on dSPN cell bodies and dendrites in the striatum causes the release of an endogenous endocannabinoid called 2-Arachidonyl Glycerol, which binds to cannabinoid 2 receptors on nigrostriatal terminals from the substantia nigra pars compacta (SNc), thereby reducing the likelihood of dopamine release. Within the figure, neurons are color-coded based on the neurotransmitter that they release, DA and 2-Arachidonyl glycerol release is represented by wavy lines, and receptors are also represented by shapes of different colors, see key. Abbreviations: GPe, globus pallidus externa.

signaling, specifically the release of the endocannabinoid 2-arachidonoylglycerol (2-AG) which then binds to $G\alpha_{i/o}$-coupled CB2 cannabinoid receptors (CB_2) located on nigrostriatal terminals [16].

Historically, selectively targeting M_4 with pharmaceutical compounds has been challenging due to a close sequence homology between the orthosteric site of muscarinic receptors.There are compounds under development that target the orthosteric site; however these efforts have yet to produce a truly selective M_4 compound agonist, but success has very recently been reported with orthosteric antagonists. This has led to an abundance of behavioral pharmacology research in which the interpretation of results are confounded by the use of non-selective muscarinic compounds, namely tropicamide. For more selective interrogation of M_4's role in the brain and behavior, the field has relied upon genetic global or conditional M_4 knockout (KO) mice. More recently, the discovery of compounds which target M_4 through allosteric sites has allowed for pharmacological agents to potentiate M_4 signaling that are truly selective and specific for M_4 [10–12,17,18]. These positive allosteric modulators and recently developed selective M_4 orthosteric antagonists have permitted the direct testing of the utility of M_4 inhibition and potentiation in preclinical models of movement disorders. This is especially exciting as M_4 modulation may be better tolerated by patients than general cholinergic therapies due to higher M_4 expression in the central nervous system and limited presence in the periphery. Of note, an M_4 allosteric modulator emraclidine, was recently tested in a double blind, placebo-controlled Phase 1B clinical trial in schizophrenia patients [19]. It is promising that the only peripheral side effect noted was a small and transient increase in heart rate and systolic and diastolic blood pressure that went away after 6 weeks of treatment. Below, we discuss the function of M_4 in the normal brain, how M_4 signaling may be altered in different movement disorders, and how this may be targeted for therapeutic benefit.

In the normal brain M_4 regulates DA, movement, and circuit plasticity

Under normal physiological conditions, evidence indicates that the net consequence of M_4 activation on movement is the suppression of locomotion [20]. M_4 also plays a role in behaviors and functions outside of the scope of this review, such as social behavior, and reward learning, (see [21,22]). This is supported by the fact that M_4 KO animals show increases in basal locomotion and in novelty-induced locomotion [20,22]. Additionally, M_4 KO mice exhibit augmented locomotor responses to D_1 agonists [20]. M_4 also modify effects of indirect DA agonists such as amphetamine. For instance, in wild-type animals M_4 positive allosteric modulators (PAM) decrease amphetamine-induced hyperlocomotion and striatal and accumbal DA release [23,24]. In sum, this work implies that M_4 deletion or blockade increases movement and DA signaling, whereas augmenting ACh binding to M_4 decreases movement and DA signaling, especially in the direct pathway.

Recent work suggests that M_4 opposes D_1 signaling specifically on dSPN terminals in the substantia nigra pars reticulata (SNr), and not on dendrites or cell bodies in the striatum, implicating brainstem ACh transmission in the regulation of the direct pathway [11]. Brainstem cholinergic terminals also likely express M_4 as autoreceptors, and some evidence suggests that they also project to the striatum [25], and the substantia nigra pars compacta [26]. It is possible also that M_4 are expressed on targets of brainstem cholinergic transmission; however, the exact role of brainstem ACh remains undetermined.

In addition to opposing D_1 signaling, M_4 also regulate dSPN plasticity in the striatum. Long-term potentiation (LTP) in the striatum occurs as a result of coactivation of N-Methyl-D-Aspartate (NMDA) glutamate receptors by corticostriatal glutamate, tropomyosin-related kinase B receptors which bind brain-derived neurotrophic factor, and cAMP signaling [27]. M_4 likely affects LTP in two ways: first through the inhibition of cAMP signaling, and second through interactions with the NMDA receptor. Prior research shows that M_4 PAM suppresses NMDA-evoked postsynaptic current influx and calcium transients in dSPNs [10] and promotes depotentiation in spike-time-dependent plasticity protocols. Furthermore, M_4 signaling promotes LTD in dSPNs. Interestingly, a system of plasticity checks and balances is built into dSPNs, as calmodulin-dependent protein kinase II, which is active in LTP, binds to the second intracellular loop of M_4 and can potentiate its activity through threonine phosphorylation [28].

Prepulse inhibition of startle is also decreased by M_4 KO. Critically, PPI is reduced in animals with increases or decreases in DA tone, indicating that M_4 loss likely leads to enhanced DAergic signaling [22]. Finally, in contrast with general anticholinergic therapies, targeting M_4 likely would not produce severe adverse effects on memory, as M_4 KO did not alter Morris Water Maze performance compared with controls [22]. Additionally, targeting M_4 has been shown to be efficacious for cognition in recent studies [29,30]. Overall, these studies provided critical evidence that M_4 regulates basal ganglia nuclei linked to several diseases and implicates that M_4 should be further studied for their role in modulating the symptoms of movement disorders.

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M_4 contribution to movement disorders

Hypo-dopaminergic disorders

Dystonia

Dystonia is a set of sporadic and genetic movement disorders associated with involuntary, repetitive muscle contractions and abnormal postures (See Figure 2 for circuit changes in dystonia). Disrupted striatal DA neurotransmission may be a unifying theme which is perpetuated by various mechanisms in different types of dystonia, as DA synthesis is impaired in DOPA-responsive dystonia (DRD), DA release is reduced in *Tor1A*-associated dystonia [31], and loss-of-function mutations in *GNAL* (which encodes $G\alpha_{olf}$, and couples to D_1 in dSPNs) prevent efficient signal transduction of DA (Fuchs et al. [32]). Some studies have also found a reduction in D_2 protein or receptor availability in animal models of DYT1 [33–36] and in humans dystonia patients [37,38]. Furthermore, other genetic dystonias which do not have direct links to DA synthesis or signaling appear to have altered DA homeostasis, suggesting that disturbed DA may be critical to dystonia pathogenesis. One possible mechanism to ameliorate this disturbed DA signaling is through targeted cholinergic modulation.

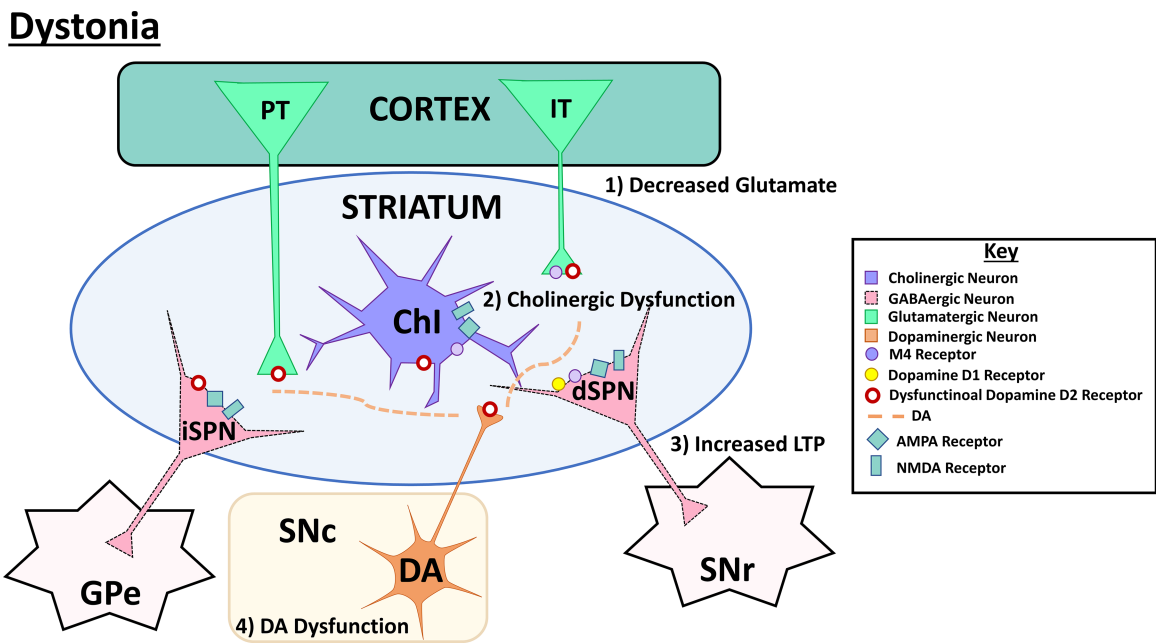

Figure 2. Circuit dysfunction in dystonia.

Circuit dysfunction in dystonia occurs due to a variety of factors including increased cholinergic tone and M_4 muscarinic acetylcholine receptor (M_4) signaling, a decrease in dopamine (DA) D_2 receptor (D_2) protein and paradoxical excitation of striatal cholinergic interneurons when DA binds to D_2. These abnormalities in D_2 signaling are conveyed in the figure by a white-filled circle with a red outline where D_2 is normally expressed. The consequences of this abnormal elevated striatal ACh release and aberrant DA signaling are listed below. (1) Corticostriatal glutamate release is increased in dystonia, likely due to a reduction in dopamine D_2 protein. Please note that corticostriatal intratelencephalic (IT) and pyramidal tract (PT) neurons synapse onto both direct pathway spiny projection neurons (dSPN) and indirect pathway spiny projection neruons (iSPN). (2) There is cholinergic dysfunction in dystonia including an increase in striatal ACh release, death of cholinergic interneurons (ChI), and changes in D2R signaling, whereby DA binding to D_2 leads to paradoxical excitation of striatal ChIs. (3) Increases in direct pathway long-term potentiation (LTP) and reductions in direct pathway long-term depression (LTD) also occur. One possible reason for direct pathway LTP increase is M_4 dysfunction in dystonia. (4) There is marked dopaminergic dysfunction in dystonia, with a reduction in D_2 protein, disrupted DA synthesis in DOPA-responsive dystonia, reduced DA release in Tor1A-associated dystonia, or loss of function mutations in GNAL dystonia, which negatively impact D_1 signaling. Within the figure, neurons are color-coded based on the neurotransmitter that they release, DA release is represented by a dashed orange line, and receptors are also represented by shapes of different colors, see key. Abbreviations: GPe, globus pallidus externa; SNr, substantia nigra pars reticulata; SNc, substantia nigra pars compacta.

Changes in DA may alter cholinergic release or signaling, which provides interesting therapeutic targets for dystonia. Currently, non-selective anti-muscarinic antagonists remain the only orally bioavailable small molecule therapeutics for dystonia. In mouse models of dystonia, increased striatal cholinergic tone may result in increased M_4 signaling at corticostriatal terminals, as well as dSPNs [24,25]. This is supported by initial studies with pharmacological inhibitors of M_4 which relieve motor dysfunction in preclinical dystonia models [18].

The increase in cholinergic tone may also suggest that M_4 autoreceptors are dysfunctional in dystonia. Death of ChIs within the first 1–2 months in certain mouse models of DYT1 linked dystonia, occurs possibly due to excitotoxicity. Death of ChIs is accompanied by reductions in choline acetyltransferase and vesicular ACh transporter [39,40]. Dendritic trees of striatal ChIs in DRD dystonia mice also show marked decreases in complexity [40,41]. These cholinergic modifications lead to changes in DA signaling in ChIs via M_4 saturation of $G\alpha_{i/o}$ signaling where increased cholinergic tone elicits paradoxical excitation of ChIs by D_2 receptors [33 42,43]. However, whether normalizing these cholinergic mediated deficits is therapeutically relevant remains untested.

Recent evidence from dystonia patients suggests that there is disrupted functional connectivity in the motor cortex which may be a compensatory mechanism to counter reduced DA signaling [44–46]. This is in accordance with observed alterations in LTD multiple dystonia models [47]. One factor that may contribute to this abnormal activity is the blunting of M_4 signaling in dystonia, as M_2/M_4 knockout mice show similar abnormalities in synaptic plasticity [48]. Perhaps, in response to the reduction or lack of DA signaling in the direct pathway, DA receptors sensitize whereas M_4 desensitize to allow for more movement. This is broadly supported by evidence that multiple types of dystonia are responsive to anticholinergic therapy, which could increase the likelihood of M_4 inhibition of LTP [49–51]. Relatedly, recent evidence shows that M_4 antagonism reverses dystonic movements in DRD mouse models and normalizes DA release *TOR1A* dystonia models [18,31]. Furthermore, M_4 are required for the effects of Trihexyphenidyl on dystonia [31]. However, the extent of efficacy of M_4 antagonism in different types of dystonia both genetic and sporadic has largely not been examined.

Parkinson's disease

PD is neurodegenerative disorder which is characterized by motor symptoms including akinesia, bradykinesia, gait difficulties, and in some patients, development of cognitive impairment or dementia (See Figure 3 for circuit changes in PD). Death of nigrostriatal DA neurons leads to low DAergic tone; whereas striatal cholinergic tone is augmented [3,52]. This increase in cholinergic tone appears to be clinically relevant, as non-selective cholinergic antagonists were originally used to treat PD and remain an additive therapy. The increase in cholinergic tone may be a result of M_4 autoreceptors on striatal ChIs becoming desensitized in PD after an increase in regulator of G-protein signaling 4 (RGS4; [53]). RGS4 is a guanosine triphosphatase-accelerating protein associated with autoreceptors, and it reduces G-protein coupling between receptors and effectors, in this case, specifically Cav2 calcium channels and potassium channels, which regulate ChI activity and ACh release.

As a result of augmented cholinergic tone, striatal M_4 signaling may be increased on dSPNs which likely contributes to LTD and motor symptoms such as bradykinesia and akinesia. Furthermore, M_4 signaling on dSPN terminals in the SNr is also likely increased in PD. In multiple preclinical lesion models, pedunculopontine nucleus cholinergic neurons become hyperactive [54–56], which could even further inhibit dSPN firing. Corticostriatal glutamate release would also be reduced by M_4 signaling. Within the cortex, there are two types of neurons that project to the striatum: the pyramidal tract (PT) neurons which innervate the ipsilateral striatum and encode information about ongoing motor activity and intratelencephalic tract (IT) neurons which innervate the striatum bilaterally and carry information related to motor planning and the environment [57–59]. IT and PT neurons synapse onto both dSPNs and iSPNs [57]. Recent studies in PD models demonstrate inhibition of PT but not IT neurons [60,61], which may contribute to the lateralized symptom profile in PD patients, where pathology is more severe on one side of the body. M_4 signaling on corticostriatal terminals of PT neurons may further exacerbate this effect as observed in preclinical models of parkinsonian motor deficits and blocking this may normalize corticostriatal transmission and alleviate some PD motor symptoms.

Interestingly, in 6-OHDA lesion models there is a loss of synaptic plasticity in SPNs at 3–4 weeks post-lesion [62], which could be due to a marked decrease in glutamate and DA signaling onto SPNs [63,64]. By 2 months post-6-OHDA-lesion, dSPNs undergo spine pruning, which is consistent with a state of LTD, which could be perpetuated by increased M_4 signaling. Finally, by 3 months post-lesion, dSPNs undergo substantial spine loss [62]. Later in disease progression, compensatory changes occur whereby dSPNs show increases in intrinsic excitability. This change may be due, in part, to simultaneous increase in D_1 sensitization paired with M_4

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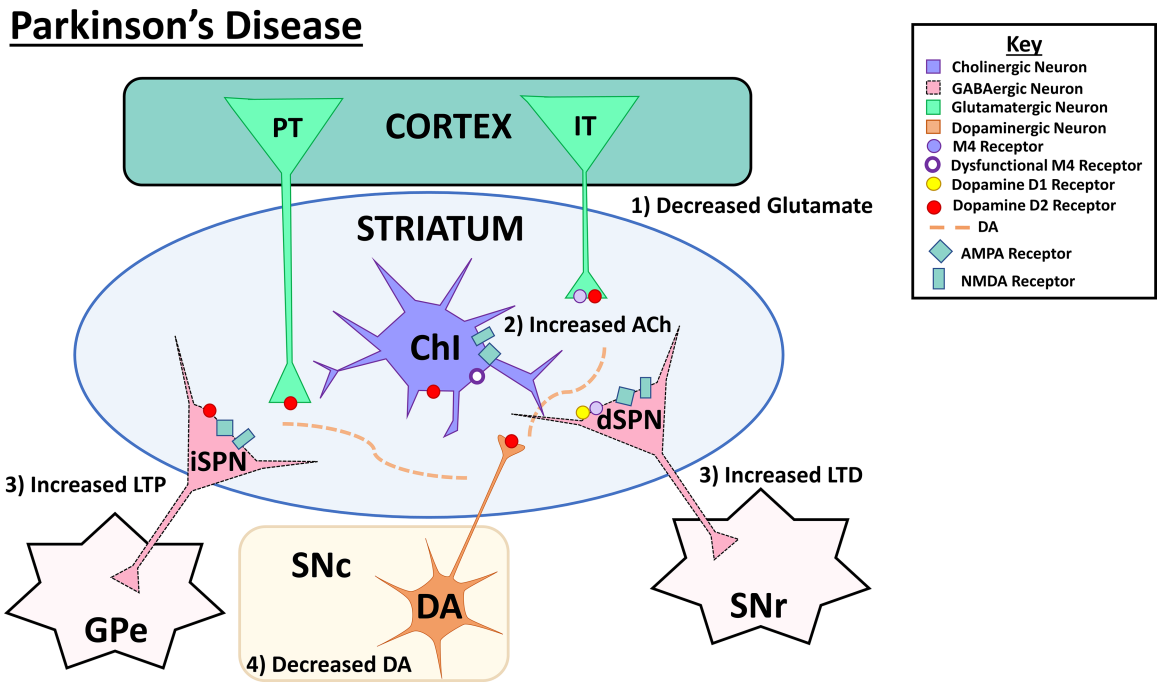

Figure 3. Circuit changes in Parkinson's disease.

There are multiple changes in synaptic plasticity and activity that occur in Parkinson's disease after death of nigrostriatal dopamine (DA) neurons. (1) There is a decrease in corticostriatal glutamate, which is partially due to increases in M_4 muscarinic acetylcholine (ACh) receptor (M_4) signaling on corticostriatal terminals. Please note that corticostriatal intratelencephalic (IT) and pyramidal tract (PT) neurons synapse onto both direct pathway spiny projection neurons (dSPN) and indirect pathway spiny projection neurons (iSPN). (2) There is an increase in striatal cholinergic tone, which is likely due to desensitization of M_4 autoreceptor as there is an increase in RGS4 within cholinergic interneurons (ChI). RGS4 is a protein which acts as a guanosine triphosphatase, decreasing Cav2 calcium channel modulation and potassium channel modification by M_4, and thus breaking M_4 modulation of cholinergic interneurons (ChI) firing and ACh release. In the figure this is represented by the M_4 receptor being filled in with white on the ChI. (3) Meanwhile an increase in M_4 signaling on dSPNs and decreased DA signaling in the striatum leads to long-term depression (LTD) in the direct pathway, thus inhibiting movement and leading to a decrease in spine density on dSPNs. At the same time, indirect pathway spiny neuron (iSPN) long-term potentiation (LTP) is increased in the absence of DA. (4) DA neurons in the substantia nigra die, reducing the amount of striatal DA. Within the figure, neurons are color-coded based on the neurotransmitter that they release, DA release is represented by a dashed orange line, and receptors are also represented by shapes of different colors, see key. Abbreviations: GPe, globus pallidus externa; SNr, substantia nigra pars reticulata ; SNc, substantia nigra pars compacta.

desensitization, which may be mediated by the progressive inactivity of calmodulin-dependent protein kinase II in the absence of LTP [28]. Despite the contribution of M_4 to directly regulating neurons and circuits shown to be altered in preclinical models of parkinsonian motor deficits as discussed above, the ability for M_4 to directly modulate the symptoms of PD remains largely unexplored with only one study directly showing that M_4 antagonism relieves bradykinesia and forelimb asymmetry in models of parkinsonian motor deficits [18].

Hyper-dopaminergic disorders

Huntington's disease

Huntington's disease (HD) is an autosomal dominant disorder caused by an expansion of the CAG triplet repeat in the huntington gene (htt) which leads to a progressive degeneration of SPNs and cortical cells (See Figure 4 for circuit changes in HD). Early in disease progression there are heightened levels of nigrostriatal DA release; whereas later in disease progression there are reduced levels of DA release [65]. There are marked cholinergic abnormalities in HD including a reduction in ChAT, and likely ACh synthesis (See Figure 4). Additionally, striatal ACh release is reduced, and ChI function is impaired. Although mixed results have

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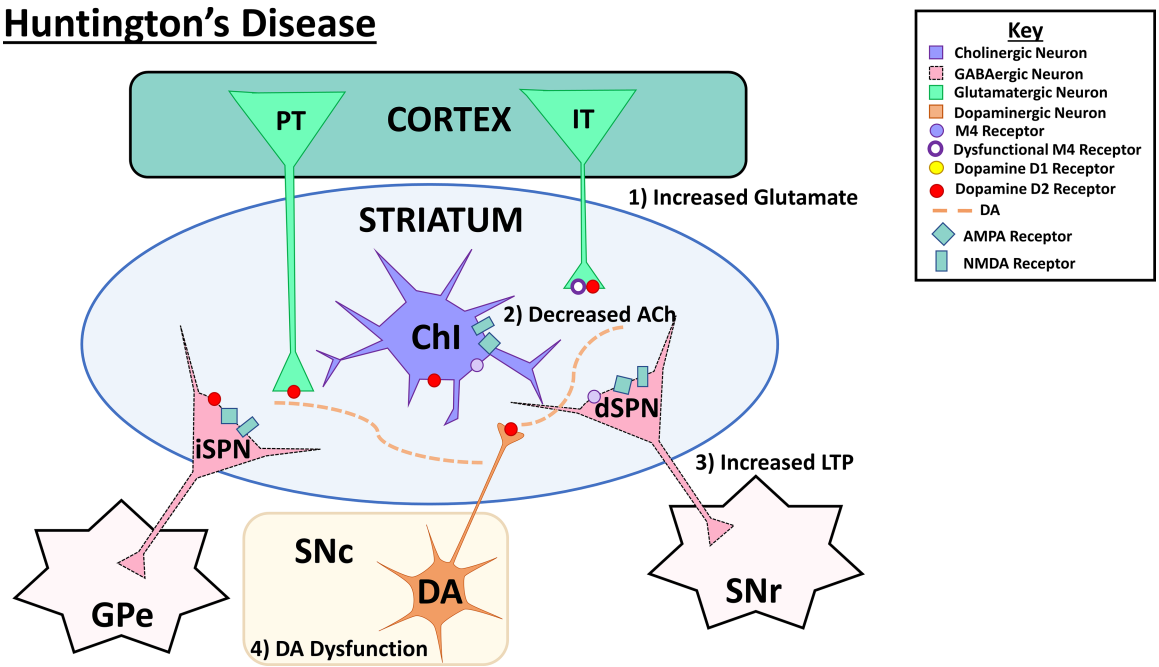

Figure 4. Circuit changes in Huntington's disease.

Circuit dysfunction in Huntington's disease largely arises due to aberrant activity of nigrostriatal dopamine neurons, corticostriatal glutamate neurons, and striatal cholinergic interneurons (ChI). (1) There is an increase in corticostriatal glutamate release, especially in intratelecephalic tract (IT) neurons. This increase in glutamate release is likely caused by a reduction in inhibitory signaling at terminal $G\alpha_{i/o}$-coupled dopamine (DA) D_2 receptors (D_2) and M_4 muscarinic acetylcholine (ACh) receptors (M_4). (Please note that corticostriatal intratelencepahlic (IT) and pyramidal tract (PT) neurons synapse onto both direct pathway spiny projection neurons (dSPN) and indirect pathway spiny projection neurons (iSPN)). (2) There is decreased ACh release in the striatum and the function of ChI is impaired. (3) dSPN long-term potentiation (LTP) is also increased due to the heightened corticostriatal glutamate release. (4) Abnormalities in DA transmission also occur, with heighted DA release early in disease progression and reduced DA release later in disease progression. Within the figure, neurons are color-coded based on the neurotransmitter that they release, DA release is represented by a dashed orange line, and receptors are also represented by shapes of different colors, see key. Abbreviations: GPe, globus pallidus externa; SNr, substantia nigra pars reticulata ; SNc, substantia nigra pars compacta.

occurred in human HD patients, some studies suggest that inhibiting the breakdown of ACh via inhibitors of acetylcholinesterase may rescue deficits in some patients. Corticostriatal drive from the intratelencephalic tract (IT) neurons to SPNs is increased, promoting dSPN LTP [66]. Intriguingly, deleting mutant htt from ChIs but not from the cortex normalized cholinergic tone and restore IT neurons to their normal state of connectivity with SPNs, suggesting that cholinergic signaling is regulating corticostriatal drive in early HD.

Underlying these changes in corticostriatal signaling may be altered M_4 activity. There is aberrant M_4-dependent plasticity in HD. In the R6/2 preclinical mouse model of HD, M_4 expression is reduced in the cortex and likely at corticostriatal synapses [67]. This reduction in expression of M_4 enhances corticostriatal glutamate release, and likely contributes to dSPN LTP and chorea. Directly targeting M_4 through selective potentiation of M_4 in presymptomatic periods normalized corticostriatal glutamate release, normalized nigrostriatal DA release, and prevented the onset of motor symptoms [68]. Thus, targeting M_4 may be beneficial early in HD progression through countering the effects of over-active corticostriatal drive through depotentiation, and possibly represents a disease modifying strategy in HD.

L-DOPA-induced dyskinesia

L-DOPA-induced dyskinesia (LID) is a side effect of the standard treatment for PD that involves the development of abnormal involuntary movements that can be extremely debilitating (See Figure 5 for circuit changes).

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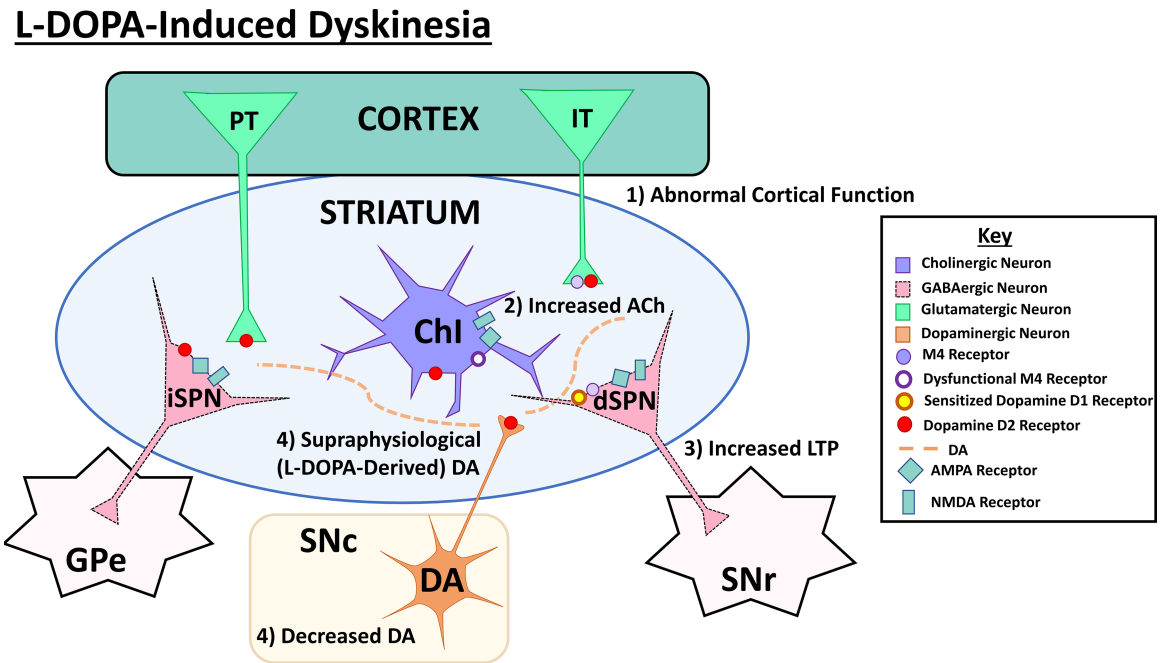

Figure 5. Circuit changes in L-DOPA-induced dyskinesia.

There are profound changes in dopamine (DA) and acetylcholine (ACh) signaling in L-DOPA-Induced dyskinesia (LID). (1) There is abnormal activity in motor cortex, with L-DOPA causing a reduction in cortical activity as compared with the parkinsonian state. At the same time there is a facilitation of glutamate signaling which occurs with LID, and higher levels of cortical activity are associated with more severe LID. (Please note that corticostriatal intratelencephalic (IT) and pyramidal tract (PT) neurons synapse onto both direct pathway spiny projection neurons (dSPN) and indirect pathway spiny projection neurons (iSPN)). (2) There is an increase in striatal ACh levels, possibly due to dysfunction of M_4 autoreceptors on cholinergic interneurons (ChI) due to an increase in RGS4. RGS4 is a protein which acts as a guanosine triphosphatase, decreasing Cav2 calcium channel modulation and potassium channel modification by M_4, and thus breaking M_4 modulation of cholinergic interneurons (ChI) firing and ACh release. In the figure this is represented by the M_4 receptor being filled in with white on the ChI. (3) There is an increase in long-term potentiation (LTP) in the direct pathway, due to sensitization of D_1 receptors, which is represented by a bold outline on the D_1 receptor. (4) Due to the death of nigrostriatal DA neurons, raphestriatal serotonin neurons convert L-DOPA into DA, and these serotonin neurons lack the cellular machinery to regulate DA release (e.g. D_2 receptors and DA transporter). This results in supraphysiological, pulsatile DA availability in the striatum following L-DOPA administration. Within the figure, neurons are color-coded based on the neurotransmitter that they release, DA release is represented by a dashed orange line, and receptors are also represented by shapes of different colors, see key. Abbreviations: GPe, globus pallidus externa; SNr, substantia nigra pars reticulata; SNc, substantia nigra pars compacta.

L-DOPA improves parkinsonian motor deficits and re-establishes dSPN LTP in animal models of disease. In the parkinsonian state, raphestriatal serotonin neurons convert exogenous L-DOPA into DA [69], but unlike nigrostriatal DA neurons, serotonin neurons lack D_2 and DA transporter, resulting in uncontrolled DA release and pulsatile DA stimulation in the striatum. Coupled with changes in dSPNs such as D_1 sensitization and increased arborization, this leads to an inability for dSPNs to be depotentiated. Additionally, striatal cholinergic tone remains elevated [3,52], suggesting that M_4 autoreceptors on ChIs are likely still dysfunctional due to increases in RGS4 [53]. Because of elevated cholinergic tone, excessive M_4 signaling may also contribute to the decoupling of striatal signaling from behavioral outcomes. Interestingly, selectively killing ChIs can attenuate LID [52]. Motor cortex activity is reduced by both acute and chronic L-DOPA treatment in both humans [70,71] and preclinical models [72], and existing data suggest that motor cortex activity is positively correlated with LID severity, and that pharmacologically inhibiting motor cortex neurons may decrease LID [72]. M_4 provide another more specific avenue by which cholinergic transmission can be normalized, corticostriatal glutamate release can be reduced, and the direct pathway can be depotentiated. Indeed, M_4 PAM VU0467154 has

been shown to reduce LID in preclinical rodent and non-human primate models [10]. This is corroborated by new evidence that chemogenetic modulation of dSPNs with modified human M_4 receptors that respond to designer drugs (hm4di) delays onset of dyskinesia, reduces the duration of dyskinesia, and attenuates total dyskinetic behavior [73]. However, follow-up studies are needed, as potentiating M_4 effects may worsen PD motor symptoms, especially in the time window of the off-period before the next dose of L-DOPA, where synaptic availability of DA is low.

Conclusion/future directions

Taken together, experimental evidence suggests that the overall role of M_4 is to modulate DA release and signaling which opposes locomotion in normal physiological states. Furthermore, M_4 show great promise as a pharmacological target for treating movement disorders symptoms through normalization of aberrant synaptic plasticity underlying movement disorders symptoms. Specifically, an M_4 PAM has already shown efficacy in lessening the severity of LID in preclinical models [10] and for normalizing DA release and preventing onset of motor symptoms in an HD mouse model [68]. M_4 antagonists reverse dystonic movements in DRD mouse models and normalize DA release in TOR1A dystonia models. Finally, M_4 antagonism relieves bradykinesia and forelimb asymmetry in preclinical mouse models of PD [18]. However, the efficacy of M_4 modulating agents to remove symptoms of movement disorders remains largely clinically untested. In particular, M_4 sensitization and desensitization, trafficking, and protein production may be altered as a compensatory mechanism in movement disorders, leading to homeostatic changes in neurons, and these changes may occur with both region and cell-specificity. Furthermore, the potential disease modifying properties of brainstem and basal forebrain cholinergic projections activating M_4 need further investigation not only for symptomatic relief, but also as disease modifying strategies as recent data from HD and Alzheimer's models suggest that cholinergic manipulations may be disease modifying. Finally, while great progress has been made in identifying the behaviors and synaptic processes of M_4 with cell type specificity in regulating basal ganglia motor circuits, this has not translated to understanding of specific behaviors that can be altered by targeting M_4 on specific neurons in animal models of disease. This will be critical to understanding the mechanism of action and the extent of efficacy of targeting M_4. Overall, M_4 represents an exciting pharmaceutical target for improvement of movement disorders symptoms, and possibly disease modification.

Perspectives

- The development of M_4 selective compounds is an exciting and important development for the investigation and treatment of movement disorders.
- M_4 both directly and indirectly counters DA transmission in the basal ganglia, and are present at multiple loci that have potential disease-modifying effects.
- Potentiation of M_4 reduces symptoms hyper-dopaminergic diseases such as HD and LID, and M_4 antagonism reduces symptoms of hypo-dopaminergic diseases such as dystonia and PD.
- Future investigations into M_4 plasticity in movement disorders are necessary. The disease-modifying effects of M_4 need to be further investigated, and specific symptoms and aberrant neuroplasticity that are improved by M_4 modulation should be defined.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

2-AG, 2-arachidonoylglycerol; ACh, acetylcholine; cAMP, cyclic adenosine monophosphate; CB_2, cannabinoid type 2 receptor; ChI, cholinergic interneuron; D_1, dopamine D1 receptor; D_2, dopamine D2 receptor; DA, dopamine; DRD, DOPA-responsive dystonia; dSPN, direct pathway spiny projection neuron; HD, Huntington's disease; hM4di, modified human muscarinic M4 receptor; Htt, Huntingtin; IT, intratelencephalic tract neuron; KO, knock-out; LID, L-DOPA-induced dyskinesia; LTD, long-term depression; LTP, long-term potentiation; M_4, muscarinic acetylcholine M4 receptor; NMDA, N-methyl-D-aspartate receptor; PAM, positive allosteric modulator; PD, Parkinson's disease; PT, pyramidal tract neuron; RGS4, regulator of G-protein signaling 4; SNr, substantia nigra pars reticulata; SPN, spiny projection neuron.

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