nature neuroscience

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Direct and indirect pathways of basal ganglia: a critical reappraisal

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The basal ganglia are subcortical nuclei controlling voluntary actions and have been implicated in Parkinson's disease (PD). The prevailing model of basal ganglia function states that two circuits, the direct and indirect pathways, originate from distinct populations of striatal medium spiny neurons (MSNs) and project to different output structures. These circuits are believed to have opposite effects on movement. Specifically, the activity of direct pathway MSNs is postulated to promote movement, whereas the activation of indirect pathway MSNs is hypothesized to inhibit it. Recent findings have revealed that this model might not fully account for the concurrent activation of both pathways during movement. Accordingly, we propose a model in which intrastriatal connections are critical and the two pathways are structurally and functionally intertwined. Thus, all MSNs might either facilitate or inhibit movement depending on the form of synaptic plasticity expressed at a certain moment. In PD, alterations of dopamine-dependent synaptic plasticity could alter this coordinated activity.

"Homines dum docent discunt" (men learn while they teach). This sentence is from the seventh letter of Seneca to Lucilius[1]. Seneca indicates that learning is a mutual process. For example, when preparing or giving a lecture on an established scientific theory, one is forced to weigh the evidence for and against this theory. Often, lecturing can require greater clarity than is necessary when discussing experiments in the laboratory. Thus, when teaching, we repeatedly analyze a theory, learning through this didactic process. For instance, teaching in a course of neuroscience and neurology, the functional anatomy of several systems and its clinical consequences seems relatively easy. Conversely, the description of the functional role of the basal ganglia (BG) in motor control and the pathophysiology of related conditions such as PD appears much more complex. Thus, when explaining the canonical model of BG organization, the teacher must either provide an oversimplification or a too complex explanation of the circuits underlying movement and related disorders.

Is this problem a simple and limited didactic dilemma, addressable with better chapters in textbooks, or does it involve a more general problem with our understanding of the real organization of the BG? Bearing out Seneca's wisdom, while teaching BG physiology and pathophysiology according to the model of direct/indirect pathways, we came to believe that the current view has some limitations. In particular, we feel that this model seems too rigid to account for recent experimental and clinical findings that have revealed the complexity of the system while simultaneously being too complex to explain the organization of the BG to a class of students. Below, we attempt to address this issue, discussing the implications of recent findings for the direct/indirect pathway model.

Before the model

Several critical anatomical and physiological descriptions of BG networks and the striatal complex were made before the elaboration of the direct/indirect model[2]. From these studies, a simple network emerged, linking cortex, striatum and output structures. Briefly, the striatal complex receives glutamatergic excitatory inputs from cortical and thalamic structures. These inputs converge in the striatum to establish synapses with both MSNs, the GABAergic output cells representing about 95% of striatal neurons[3], and aspiny GABAergic and large cholinergic interneurons[4]. Other neuromodulatory inputs[5], most prominently dopaminergic inputs from the substantia nigra pars compacta (SNpc)[5], reach the striatum. These dual glutamatergic and dopaminergic projections converge onto dendritic spines of the same MSN[6]. In addition, striatal interneurons receive both glutamatergic and dopaminergic inputs, and most of them synapse onto MSNs, representing a link between neuronal inputs from striatal afferents and striatal projecting neurons[7].

The classical model of direct and indirect pathways

The canonical view of the interaction between glutamatergic and dopaminergic neurotransmission in the striatum, which lies at the heart of the direct/indirect pathway model, originated in seminal papers that hypothesized a dual organization of the striatum and of BG outputs[8,9]. According to this model, cortical activation produces a release of glutamate that activates MSNs projecting to the substantia nigra pars reticulata (SNpr) and the globus pallidus pars interna (GPi) (the striato-nigral output neurons representing the direct pathway; **Fig. 1**). MSNs are GABAergic cells; thus, they exert an inhibitory action on neurons of the SNpr that are also GABAergic. This inhibition of the SNpr leads to a disinhibition of the thalamic glutamatergic neurons, which receive SNpr input and project to the cortex. The behavioral result of this chain of events is locomotor activation/movements.

Conversely, activation of striato-pallidal MSNs, which project indirectly to the SNpr via the globus pallidus pars externa (GPe) and the subthalamic nucleus (STN) (indirect pathway), inhibits the

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Received 14 February; accepted 21 May; published online 28 July 2014; doi:10.1038/nn.3743

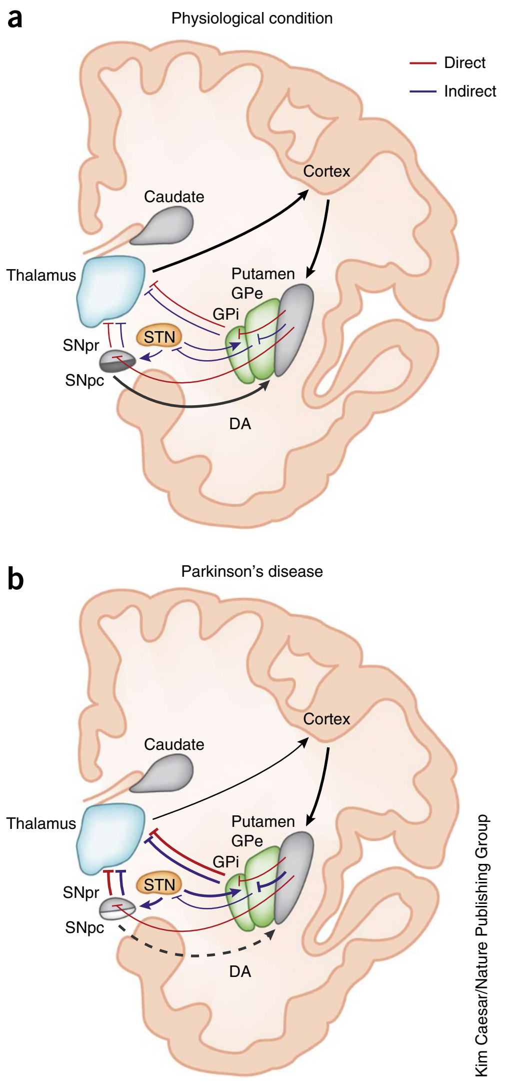

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Figure 1 Schematic representation of the direct/indirect pathway classical model in the physiological condition and in Parkinson's disease. (**a**) In the physiological condition, DA arising from the SNpc is thought to activate D1-expressing striatal MSNs of the direct pathway (red lines) and to inhibit D2-expressing striatal neurons of the indirect pathway (blue lines). The output nuclei GPi and SNpr project to the thalamus, which in turn sends efferents that complete the cortico-basal ganglia-thalamo-cortical loop. (**b**) In Parkinson's disease, degeneration of nigral neurons reduces DA receptor stimulation in striatal MSNs. The imbalance between direct and indirect pathways results into abnormal activation of output nuclei and over-inhibition of thalamic neurons projecting to the cortex.

GABAergic neurons of the GPe, leading to a disinhibition of the glutamatergic neurons of the STN. The increased discharge of these excitatory STN neurons in turn activates the SNpr GABAergic neurons projecting to the thalamus. Ultimately, this effect results in the reduction of locomotor activity and movement (**Fig. 1**).

In addition to their distinct projections, MSNs of the direct and indirect pathway are characterized by the differential expression of dopamine (DA) receptors. D1 DA receptors are expressed by direct pathway MSNs, whereas D2 receptors are expressed by indirect pathway MSNs. These two receptors are associated with distinct G proteins that are linked to different intracellular signaling pathways and lead to different biochemical responses following DA receptor activation. This neurochemical segregation is considered to be further support for a dichotomous effect of the activation of the direct and indirect pathways[10,11].

Experimental consequences of the model

The direct/indirect pathways model has been widely used to explain experimental findings, build models of BG disorders, and explain therapeutic effects of both pharmacological and neurosurgical treatments. Distinct, and even opposite, roles of these two pathways in regulating several physiological functions involving the BG, such as basal locomotor activity and motor responses to drugs of abuse and antipsychotic agents, have even been postulated[12,13]. In particular, the selective loss of the striatal signaling protein DA- and cAMP-regulated phosphoprotein 32 kDa (DARPP-32) in direct pathway MSNs reduces basal and cocaine-induced locomotion, and, in a rodent model of PD, abolishes dyskinetic behavior in response to levodopa (L-DOPA), a DA precursor that is widely used as a form of therapy for PD. Conversely, a loss of DARPP-32 in indirect pathway MSNs augments locomotor activity and reduces cataleptic response to the antipsychotic drug haloperidol[12].

D1 striatonigral and D2 striatopallidal MSNs have typically been considered as homogeneous populations regarding their somatodendritic morphology, although neurochemical differences have been found in these neuronal subtypes[2,14]. In fact, SNpr-projecting MSNs express substance P, dynorphin and D1 DA receptors, whereas GPe projecting MSNs express enkephalin and D2 DA receptors. The introduction and use of D1 and D2 bacterial artificial chromosome (BAC) transgenic mice[13] to distinguish MSNs without *post hoc* analyses led to the identification of further possible differences between these two populations. Electrophysiological studies[15,16] found differences in the excitability of striatal D1 and D2 MSNs, with D2 MSNs consistently firing at higher frequencies, as well as differences in resting membrane potential, input resistance and rheobase current. Moreover, experiments using two-photon laser-scanning microscopy in identified MSNs showed that the dendrites of D2 MSNs are more excitable than those of D1 MSNs, and that DA depletion augments this asymmetry[17]. Notably, three-dimensional reconstructions revealed a significantly greater total dendritic length of D1 versus D2 MSNs, suggesting that dendritic anatomy might contribute to differences in MSN excitability[18]. Finally, striatal DA denervation has been found to reduce spines and glutamatergic synapses, potentially via dysregulation of a Ca^{2+} channel implicated in the pathophysiology of PD, on striatopallidal, but not striatonigral, MSNs[19].

The introduction of D1 and D2 BAC transgenic mice has also provided new results regarding features of striatal synaptic plasticity and its possible functional implications. Although previous studies have demonstrated the expression of activity-dependent long-term depression (LTD) in the large majority of MSNs, suggesting an absence of neuronal segregation between the two pathways[20–23], BAC-mediated targeting of direct and indirect pathways yields a different result. Kreitzer and Malenka[16] showed a selective D2 receptor activation–dependent LTD in MSNs of the indirect pathway. Notably, this form of synaptic plasticity was absent in PD rodent models and was rescued by D2 receptor stimulation. To determine whether synaptic plasticity could be unidirectional in D1 and D2 receptor–expressing MSNs, spike timing–dependent plasticity was also investigated in brain preparations from DA receptor BAC transgenic mice. This study showed that, although DA has a complex and complementary role in these two types of MSNs to ensure bidirectional plasticity in physiological conditions, this role is altered in mouse models of PD and only unidirectional changes in plasticity occur[24].

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More recently, optogenetic techniques allowed the identification of specific cell types *in vivo* through optical stimulation of recorded cells or optical monitoring of the activity of neurons of a specific cell type. This approach has permitted the measurement of neural activity-dependent fluorescence changes from specific types of neurons of the BG in behaving animals. A study using *in vivo* optogenetic methods showed that excitation of D1 and D2 receptor–expressing MSNs acts bidirectionally on locomotion[25]. Notably, bilateral excitation of indirect pathway MSNs elicited a parkinsonian-like state, characterized by freezing, bradykinesia and decreased locomotor initiation. Conversely, activation of direct pathway MSNs reduced freezing and increased locomotion. Moreover, in a rodent model of PD, activation of direct pathway reversed freezing, bradykinesia and deficits of locomotor initiation[25].

An interaction between the direct and indirect pathways in action selection has been postulated, suggesting an integration between these pathways in producing coordinated behavior, both in terms of motor output and in fine temporal patterning of neural activity[26,27]. Indeed, these interpretations of the classic direct/indirect model predict that both pathways are active during movement selection and that the integration of the two outputs arbitrates between the ultimate selection of motor programs based on outcomes obtained from specific actions. This was highlighted in an interesting optogenetic study showing that the stimulation of the direct pathway in one hemisphere increases the likelihood of choosing the contralateral action according to action value, whereas indirect pathway stimulation has the opposite effect, effectively decreasing the action value[28].

The selective activation of the direct/indirect pathway is a possible critical aspect of the synaptic mechanisms implicated in reinforcement and punishment. Given that reinforcement maintains or increases, whereas punishment decreases, the probability of specific behaviors, alterations of these processes could be involved in psychiatric disorders[29] and drug addiction[30]. The striatum, in fact, has been highly implicated in both reinforcement and punishment processes. A recent study investigated the hypothesis that the direct (indirect) pathway mediates reinforcement (punishment). According to this hypothesis, optogenetic activation of D1-expressing neurons causes persistent reinforcement, whereas activation of D2-expressing neurons induces transient punishment[31]. Striatal DA levels, by acting in a distinct manner on either the direct or the indirect pathway, are critical in rewarding and aversive learning, as well as in drug addiction. The direct pathway seems to be involved in reward learning and cocaine sensitization, whereas the indirect pathway is implicated in aversive behavior[32]. Consistent with this latter observation, it has also been reported that a transient disruption of indirect pathway MSNs activity facilitates behavioral sensitization, whereas the decrease of excitability of direct pathway MSNs impairs the persistence of behavioral sensitization following drug exposure[33]. Findings suggesting a distinct role of the direct/indirect pathways in reward, punishment and behavioral sensitization might be relevant both for psychiatric diseases, such as depression and obsessive-compulsive disorder, and impulse control disorders in PD patients. This latter condition is a DA dysregulation syndrome, frequently observed in PD patients assuming DA replacement therapy, and causing pathologic gambling, hypersexuality, compulsive shopping, compulsive eating, excessive engagement in hobbies and punding[34].

Clinical consequences of the model

The direct/indirect pathway model has been of great importance in the interpretation of the experimental and clinical findings obtained in animal models and PD patients following pallidotomy, subthalamotomy and deep brain stimulation (DBS) of the STN, as well as of other BG nuclei (**Fig. 1**). Unilateral GPi pallidotomy improved all of the cardinal motor signs of PD, including tremor, rigidity, bradykinesia, abnormal gait and balance. In addition, L-DOPA–induced dyskinesias were markedly improved. Although the greatest improvement occurred on the side contralateral to the lesion, significant ipsilateral improvement was also observed for bradykinesia, rigidity and dyskinesias[35].

The important clinical observation that lesions of the STN, a key node of the indirect pathway, abolish the cardinal features of PD contributed to a renaissance in the use of surgical approaches in the treatment of PD. Although bilateral subthalamotomy improves symptoms in advanced PD[36], this clinical effect seems to be variable, probably depending on the location and volume of the lesions. Conversely, several clinical studies have clearly shown that the administration of high-frequency continuous electrical stimulation to the STN through a surgically implanted device reduces motor symptoms and L-DOPA–related motor complications in PD patients[37]. Clinical trials demonstrated the superior efficacy of neurostimulation over best pharmacological management both in patients with advanced PD[38] and in patients with early motor complications[39]. The successful application of DBS in PD, as a consequence of the pathophysiological theory of the direct/indirect pathway in BG disorders, led to the application of this technique in several medication-refractory hyperkinetic movement disorders, such as tremor and dystonia, as well as in psychiatric diseases, such as obsessive-compulsive disorder[40].

An imbalance in the activity of striatal direct and indirect pathway MSNs has also been postulated in Huntington's disease (HD), a progressive fatal neurological condition caused by an expansion of trinucleotide CAG repeats that leads to striatal degeneration[41]. An electrophysiological study in mouse genetic models of HD has suggested that there are differential and complex imbalances in glutamate and DA modulation in direct and indirect pathway MSNs during HD progression[42]. In particular, hyperactive behavior at the early stage could be explained by augmented glutamate activity and DA tone in direct pathway MSNs, whereas the hypokinesia observed during advanced stages could be explained by a reduced input to these neurons[42].

Experimental evidence beyond the model

Despite the experimental and clinical findings described, which support the direct/indirect pathway model, the functional relevance of these two pathways in motor generation and control is still a matter of debate. In fact, in contrast with the classical model suggesting opposing roles of the two pathways, it has recently been demonstrated that optogenetic activation of striatal direct and indirect pathway projection neurons produces different cellular responses in SNr neurons, with stimulation of each pathway eliciting both excitations and inhibitions[43]. Moreover, experimental findings have suggested a coordinated activation of both pathways during action selection. Thus, it is possible that the coordinated activity of the direct and indirect pathways is critical for the appropriate timing and synchrony of BG circuits during movement.

In particular, a recent study from Cui and colleagues[44] challenges the classical view of BG function, providing an alternative explanation for understanding the origin of motor symptoms in BG disorders. The authors developed an *in vivo* method to measure direct/indirect pathway MSN activity that uses Cre-dependent viral expression of the genetically encoded Ca^{2+} indicator GCaMP3 in the dorsal striatum of D1-Cre (direct pathway specific) and A2A-Cre (indirect pathway specific) mice and uses fiber optics and time-correlated single photon counting in mice performing an operant task. Taking advantage of

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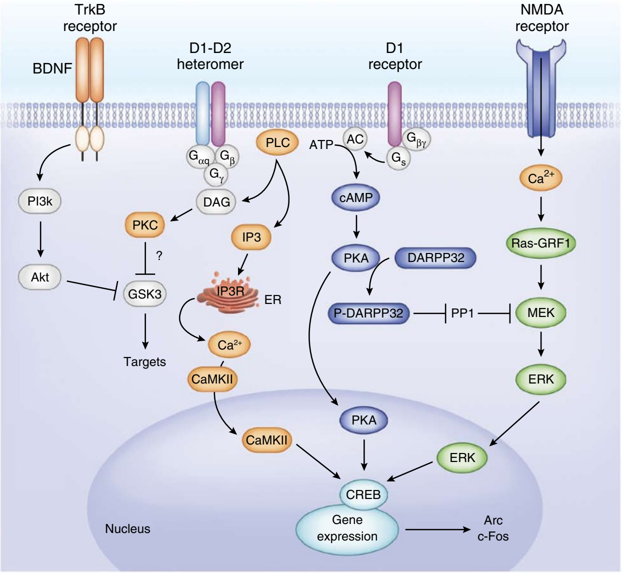

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Figure 2 Signaling pathways downstream of D1-like and D1-D2 heteromer receptor activation. Increased intracellular calcium levels, activation of calcium/calmodulin-dependent protein kinase type II (CaMKII) are mechanisms that are triggered by the activation of D1/D2 heteromers. Activation of the G-coupled D1-D2 heteromer induces a phospholipase C (PLC)-dependent calcium release, resulting in the activation of CaMKII and its translocation to the nucleus. CaMKII then induces cAMP responsive element binding protein (CREB) phosphorylation and gene expression. In addition, dopamine-induced D1-D2 heteromer activation can phosphorylate and inactivate glycogen synthase kinase-3 (GSK3). The phosphorylation state of GSK3 can be also regulated by BDNF-induced activation of TrkB receptor. Dopamine D1-like receptor can induce two different signaling pathways. Dopamine D1 receptor directly modulates NMDA receptor activation by Gβγ proteins. Moreover, by acting on cAMP level increase, the D1 receptor activation induces the activation of protein kinase A (PKA), which can translocate to the nucleus and act directly on CREB. PKA phosphorylates DARPP-32, inducing the disinhibition of the NMDA/Ras-GRF1/ERK pathway, which finally targets intranucleous CREB protein. All these downstream pathways result in the transcriptional activation of several genes. AC, adenylyl cyclase; Arc, activity-regulated cytoskeleton-associated protein; DAG, diacylglycerol; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinases; IP3, inositol 1,4,5-trisphosphate; MEK, mitogen-activated protein kinase; PKC, protein kinase C; P-DARPP-32, phosphorylated dopamine- and cAMP-regulated phosphoprotein 32 kDa; PP1, protein phosphatase 1; PI3k, phosphatidylinositide 3-kinases; Ras-GRF1, Ras-guanine nucleotide-releasing factor 1.

this innovative approach, the authors found that neural activity in both direct and indirect pathway MSNs was transiently increased when animals initiated actions. Conversely, this concomitant firing increase in both pathways was never observed when the animals were inactive. Thus, it could be assumed that activation of MSNs from both pathways in one hemisphere preceded the initiation of contraversive movements. These data on direct/indirect pathways are in apparent contrast with recent studies showing that optical activation of indirect pathway MSNs decreases locomotion[25] and that either ablation or disruption of the function of indirect pathway MSNs increases locomotion[12,45]. Thus, although optogenetic techniques have permitted the identification and the activation of specific cell types *in vivo*, these approaches might still have some technical limitations that bias functional interpretation of the obtained results. In particular, *in vivo* optogenetic imaging might not be ideal for exploring the activity of subcortical structures in freely moving animals, as there are limitations in penetration depth.

Another important issue concerning the model of motor control is how activity in cortical networks regulates direct and indirect pathways. Recently, it has been found that cortical information about motor planning and choice, conducted by intratelencephalic and pyramidal tract neurons of the motor cortex, is directed to both direct and indirect pathway MSNs[46]. This latter observation strongly supports the possibility that the two pathways act in conjunction to initiate movements as postulated by Cui and colleagues[44].

The use of D1 and D2 BAC transgenic mice to distinguish between direct and indirect pathway MSNs has also been recently questioned by experimental findings showing that these animals might show some functional alteration in comparison with wild-type mice, possibly leading to phenotypic alterations. In particular, a study using homozygous D2 eGFP mice, and based on behavioral, electrophysiological and molecular characterization, found that mice expressing eGFP through the BAC vector are not comparable with the wild-type littermates, as they overexpress D2 receptors[47]. Support for caution in the interpretation of data resulting from the use of eGFP transgenic mice is also provided by a study that combined substance P and adenosine A2A receptor immunohistochemistry (selectively expressed in direct and indirect pathways, respectively)[48,49] to identify neurons of the two pathways in both eGFP transgenic mice and control animals. Using this technique, Bagetta and colleagues found DA-dependent LTD in MSNs of both pathways in control mice, supporting the results of the original studies showing that this form of plasticity is expressed in both pathways. Surprisingly, D1 eGFP transgenic mice showed a lack of LTD in D1-expressing MSNs and showed behavioral alterations[48]. These findings suggest caution in the use of BAC mice targeting DA receptors, as genetic manipulation in these animals might result *per se* in behavioral and electrophysiological phenotypic abnormalities. Nevertheless, two other recent studies have analyzed BAC transgenic mice, suggesting that, although it is important to screen new transgenic mouse lines for abnormal behavior and physiology, these BAC transgenic lines still represent useful tools for analyzing behavior and synaptic plasticity[50,51]. Thus, this question remains open.

The direct and indirect pathways are often described not only as functionally opposing, but also as anatomically segregated. However, a recent study reviewing single-cell tracing studies in rats found that about one-third of MSNs projected exclusively to the GPe (pure indirect pathway), whereas a small minority (3%) projected only to the SNr or entopeduncular nucleus (EN) (pure direct pathway)[52]. 60% of labeled neurons projected to the SNr/EN and possessed collateral terminal fields in the GPe. Given that these GPe collaterals may have the ability to bridge the direct and the indirect pathways, they have been named bridging collaterals. Regulating the extent of bridging collaterals could be a mechanism by which the direct pathway modulates the indirect pathway, thereby affecting the behavioral balance maintained in concert by both pathways. The demonstration of these bridging collaterals might also have a strong clinical implication, as they are regulated not only by endogenous DA via the activation of D2-like DA receptors, but also by antipsychotic agents blocking D2-like receptors, such as haloperidol, a drug widely used in schizophrenia[52]. Consistent with this study, the occurrence of bridging collaterals of striatal outputs has already been hypothesized both in the rat[53] and in the monkey[54,55].

An anatomical study has reported axonal collateralization of striatofugal cells in non-human primates showing coexpression of D1-like and D2-like receptors, as well as of the different opioid peptides[56]. In particular, the authors demonstrated that neurons projecting to the GPi show immunolabeling for D1 and D2 receptors, rather than just D1 receptors, as classically viewed[56]. Similarly, striatal neurons projecting to the GPe show immunolabeling for both D1 and D2 receptors, rather than just D2 receptors[56].

Heteromers of dopamine receptors as a possible molecular cross-talk between direct and indirect pathways

In recent years, multiple levels of cross-talk between direct and indirect pathways have been revealed. Accordingly, a first level of interaction is represented by the molecular cross-talk between heteromeric D1-like and D2-like DA receptors[57,58]. Moreover, interactions between these pathways, represented by retrograde messengers and nitric oxide (NO), mediate a biochemical cross-talk, whereas the synaptic cross-talk is exerted by distinct classes of striatal interneurons. These multiple levels of interactions alter the rigid rule of the separation between the two systems.

Increased intracellular Ca^{2+} levels, activation of CaMKII and release of brain-derived neurotrophic factor (BDNF) are mechanisms triggered by the activation of D1/D2 heteromers (**Fig. 2**)[57,58]. These mechanisms are also required for striatal physiological and activity-dependent forms of synaptic plasticity[59–61], as well as for those observed following the onset of L-DOPA–induced dyskinesia in models of PD[62] and chronic drug abuse[63]. However, the possible involvement of D1/D2 heteromers in these events is still unclear, as these heteromers occur more frequently in the ventral than in the dorsal striatum[57,58]. Future studies are necessary to convincingly demonstrate the function of heteromers in native expression systems and their distinct signal transduction coupling.

Endocannabinoid system as a biochemical cross-talk between direct and indirect pathways

Endogenous molecules such as endocannabinoids (eCBs) and NO modulate the activity of MSNs by non-canonical modes, as well as that of their afferent and efferent connections, and represent an additional biochemical substrate for the cross-talk between direct and indirect pathways. Retrograde signaling is the principal mode by which eCBs mediate short- and long-term forms of plasticity both at excitatory and inhibitory synapses and interacts with dopaminergic system[64–66]. The role of eCBs in the control of LTD of MSNs is another major issue of discussion of great relevance to the direct/indirect pathway model. Lovinger's group showed for the first time that the induction of striatal LTD is dependent on activation of the CB1 cannabinoid receptor. In fact, LTD was facilitated by blocking cellular eCB uptake. The endocannabinoid necessary for striatal LTD is thus likely to be released postsynaptically as a retrograde messenger demonstrating a new role for eCBs in the induction of LTD in a circuit necessary for habit formation and motor control[65,67]. Although this effect was observed in most of the neurons, suggesting a lack of segregation in a specific pathway, the inhibition of glutamate release by retrograde endocannabinoid signaling was frequency dependent and D2 receptor mediated[68]. Notably, postsynaptic blockade of eCB membrane transport altered eCB release and LTD in the large majority of MSNs[69].

In a seminal study, Wang and colleagues[70] investigated how the induction of striatal LTD, which in the original studies[20–23] was observed in most MSNs, could depend on D2 dopamine receptors localized only in the postsynaptic membrane of a single subclass of MSNs. In fact, if this was true, LTD should be inducible in neurons from only one of the two projection systems of the striatum, as reported in the study by Kreitzer and Malenka[16]. Using transgenic mice in which neurons that contribute to these two systems were identified, Wang and colleagues demonstrated that this was not the case. They raised the idea that cholinergic interneurons, also defined as tonically active interneurons, are critically involved in this D2 dependence of LTD (**Fig. 3**). In fact, activation of D2 receptors induces pauses in the activity of these interneurons, reducing the release of acetylcholine (ACh) and relieving the inhibitory cholinergic tone on MSNs expressing M1 muscarinic receptors[70]. Given that activation of M1 receptors suppresses L-type Ca^{2+} currents, the reduced cholinergic tone disinhibits MSNs, promoting production of eCBs and LTD. Consistent with this hypothesis, application of the M1 antagonist pirenzepine reduces baseline corticostriatal glutamatergic transmission[70,71]. Moreover, this effect, induced by the activation of M1 receptors, is blocked by a CB1 receptor antagonist[70]. These observations suggest that, although the eCBs system is critical for the biochemical cross-talk between direct and indirect pathways, the cholinergic interneuron represents the cellular substrate for the synaptic cross-talk between the two classes of MSNs (**Fig. 3**). A more recent study, using immunohistochemical characterization of substance P–positive (direct pathway) and A2A receptor–positive (indirect pathway) MSNs, confirmed that D2-dependent LTD is present in both classes of MSNs[48].

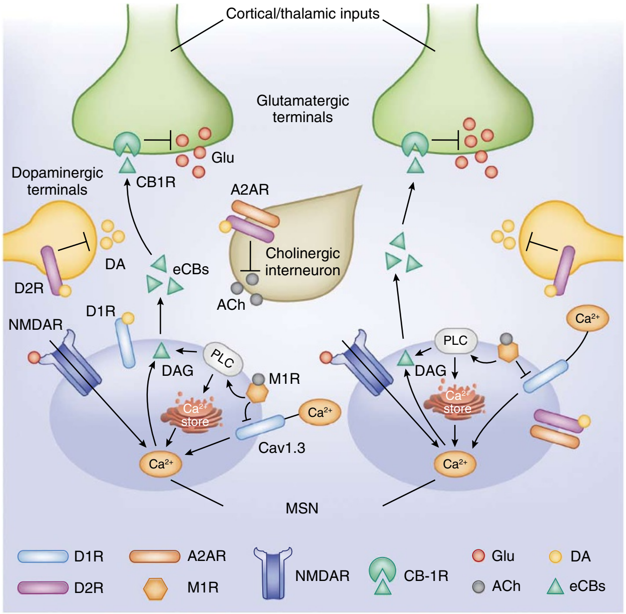

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Figure 3 Role of MSNs and cholinergic interneurons in the production and functions of endocannabinoids in the striatum. Striatal cholinergic interneurons project to both MSNs expressing D1-like and MSNs expressing D2-like dopamine receptors. The combined activation of both A2A and D2 receptors on cholinergic interneurons decreases the release of ACh. The decreased levels of ACh on the M1 muscarinic receptors located on the synaptic sites of D1 and D2 receptor–expressing MSNs reverses the blockade of the L-type calcium channels. The increase in intracellular calcium concentration might in turn trigger endocannabinoid release at the postsynaptic sites of both D1 and D2 receptor–expressing MSNs, thereby depressing glutamatergic synaptic transmission of both the direct and indirect pathways. The NMDA receptors contribute in both D1- and D2-expressing MSNs to the intracellular calcium increase and to the resulting endocannabinoid release. A2AR, adenosine 2A receptor; Cav1.3, L-type calcium channel; CB1R, endocannabinoid receptor.

Profound modifications in eCB signaling after DA depletion occur in experimental models of PD and in patients suffering from the disease[72,73]. In a PD model, striatal levels of anandamide (AEA), an endogenous cannabinoid neurotransmitter, are increased[74]. This molecular change is associated with increased spontaneous

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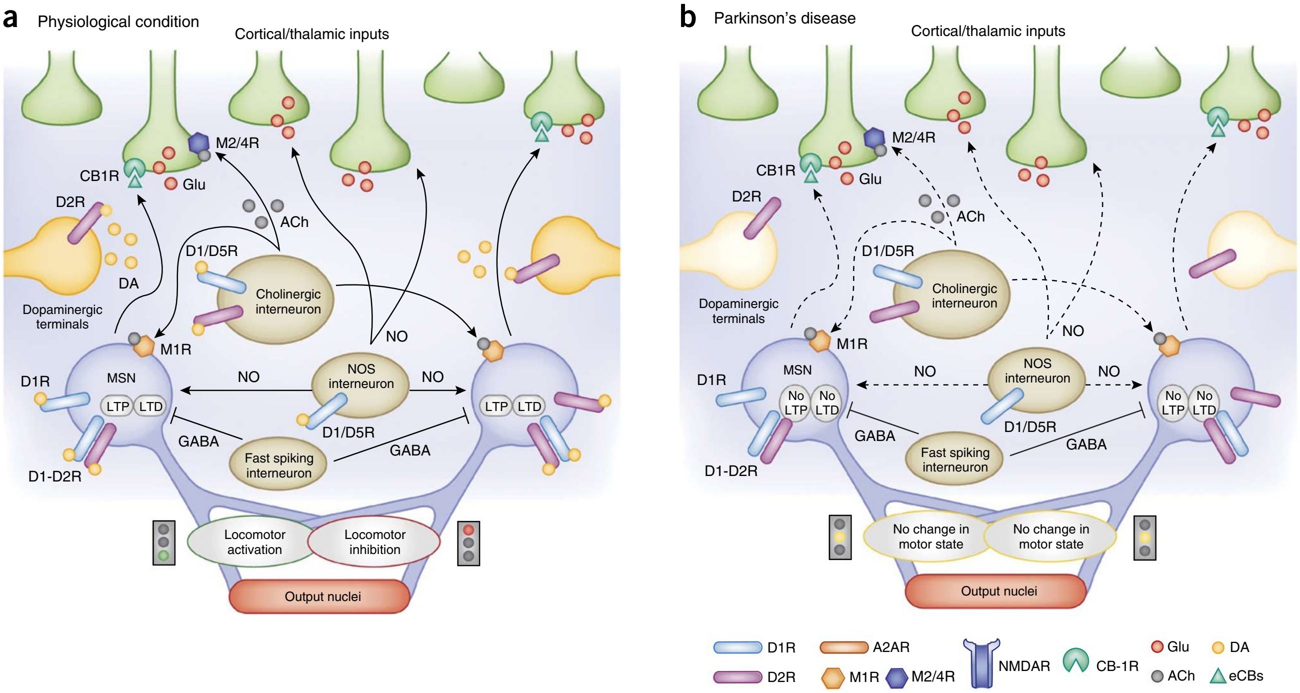

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Figure 4 Integrative hypothesis for the role of striatal circuits in controlling motor activity in the physiological condition and in Parkinson's disease. (**a**) Glutamatergic inputs originating from both the cortex and the thalamus release glutamate onto striatal neurons. Dopaminergic terminals, originating from the substantia nigra pars compacta, release dopamine onto MSNs and different subtypes of striatal interneurons. In particular, three main subtypes of striatal interneurons are implicated in the feedforward and parallel control of striatal circuits. Cholinergic interneurons release ACh acting on both presynaptic glutamatergic terminals and postsynaptic MSNs; these interneurons also respond to dopamine via D1/D5 and D2 receptors. NOS-positive interneurons produce NO, acting as a retrograde messenger as well as on MSNs facilitating LTD at the postsynaptic level. Fast-spiking interneurons release GABA on MSNs, providing a parallel inhibitory system that controls both direct and indirect pathway MSNs. MSNs can express either D1-like or D2-like receptors, as well as D1-D2 heteromeric receptors. eCBs released from MSNs can act as retrograde messengers on CB1 cannabinoid receptors located on glutamatergic terminals. The induction of either LTP or LTD in MSNs regulates the striatal control on output structures and motor activation/inhibition. (**b**) The advanced phase of Parkinson's disease is caused by a severe dopamine denervation that leads to the complete loss of striatal synaptic plasticity. Under this condition, both LTD and LTP of MSNs are lost. As a consequence of the loss of these forms of plasticity, variations of output signals from the striatum are absent and no change in the motor state can be induced. Dopamine denervation also alters the physiological activity of striatal interneurons as well as the neurochemical signals that originate from these cells and influence the activity of MSNs. A2AR, adenosine 2A receptor; CB1R, endocannabinoid receptor; DA, dopamine; D1R and D2R, dopaminergic receptors; M1R and M2/4R, muscarinic receptors.

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glutamatergic activity recorded from the large majority of MSNs and is reversed by L-DOPA treatment, making a clear segregation in the parkinsonian state also unlikely[74,75].

eCB-dependent synaptic plasticity of MSNs could represent a synaptic mechanism for the formation of persistent drug-related habits. In particular, the dorsal striatum might be implicated in the shift from casual drug use to compulsive drug use and addiction[76]. Consistent with this view, it has been observed in a mouse model of cannabinoid tolerance that persistent activation of the eCB pathway impairs LTD in MSNs[77].

NOS-positive and cholinergic interneurons: a synaptic cross-talk between direct and indirect pathways

NO was identified as a biological intercellular messenger more than 20 years ago and has been implicated in synaptic transmission and plasticity, as well as in neurodegeneration[78]. In the striatum, NO is produced by a subclass of GABAergic interneurons. In fact, in this structure, three neurochemically distinct subtypes of GABAergic interneurons have been distinguished: fast-spiking interneurons expressing the calcium-binding protein parvalbumin, interneurons expressing the calcium binding protein calretinin, and a third class of interneurons showing low-threshold spikes and coexpressing neuropeptide Y, somatostatin and nitric oxide synthase (NOS) (**Fig. 4**)[7]. Striatal NO-producing interneurons are important for the regulation of corticostriatal synaptic transmission and motor behavior (**Fig. 3**). Moreover, these interneurons can also show distinct forms of synaptic plasticity in response to different patterns of stimulation[79]. Striatal NO synthesis is stimulated by concomitant activation of glutamate and D1-like DA receptors, and this gas diffuses into the dendrites of MSNs containing high levels of NO receptors called soluble guanylyl cyclases (sGC). NO-mediated activation of sGC leads to the synthesis of the second messenger cGMP[80]. Electrophysiological experiments have shown that NOS inhibitors prevent LTD induction[81,82]. One prominent molecular target of NO is the striatally enriched sGC, suggesting the possibility that activation of this enzyme and subsequent cGMP formation is sufficient to induce LTD. Accordingly, it has been shown that the cGMP phosphodiesterase inhibitor zaprinast and the intracellular application of cGMP itself can induce LTD during low-frequency synaptic activation[81].

The role of the NO/cGMP pathway in corticostriatal LTD induction has also been investigated in a rat model of parkinsonism and L-DOPA–induced dyskinesia to test the possibility of targeting striatal phosphodiesterases to reduce involuntary movements caused by chronic treatment with this drug[82]. L-DOPA–induced dyskinesia was associated with the loss of LTD expression at glutamatergic striatal synapses onto both classes of MSNs. Inhibitors of phosphodiesterases rescued the induction of this form of synaptic plasticity via a mechanism requiring the modulation of intracellular cGMP levels.

This effect on synaptic plasticity was associated with a significant reduction of abnormal dyskinesias following intrastriatal injection of phosphodiesterase inhibitors[82]. Thus, drugs selectively targeting phosphodiesterases can ameliorate L-DOPA–induced dyskinesia, possibly by restoring physiological synaptic plasticity in MSNs of both direct and indirect pathways.

NO is also implicated in the pathophysiology of brain ischemia, and endogenous DA, via the activation of D1/D5 receptors expressed in striatal NOS-positive interneurons, seems to amplify this event. The D1-like receptor antagonist SCH-23390 prevented post-ischemic long-term potentiation (LTP) in all recorded MSNs. Immunofluorescence analysis confirmed the induction of post-ischemic LTP in both substance P–positive, (putative D1 receptor expressing) and adenosine A2A receptor–positive (putative D2 receptor expressing) MSNs[83]. Thus, in conjunction with the synaptic cross-talk between the direct and indirect pathways, represented by striatal GABAergic and cholinergic interneurons[7,14], endogenous striatal eCBs and NO constitute two systems that influence both pathways in parallel (**Fig. 3**).

Clinical observations beyond the model

The hypothesis that GPi and STN neurons are hyperactive in the parkinsonian state is supported by studies showing a reduction of parkinsonian symptoms following lesions of the GPi or STN in monkeys and patients with PD[84–86]. However, another study found that, in PD patients, apomorphine, a nonselective D1- and D2-dopamine receptor agonist, significantly decreased the firing rates of GPi neurons at doses sufficient to produce an ON state, but did not change the overall firing rate of STN neurons[87]. This latter finding suggests that the apomorphine-induced reduction of parkinsonian symptoms is not solely the result of a decrease in overall activity in the GPi or STN neurons, as predicted by the direct/indirect model of BG, but requires alternative interpretations.

There is also a simple observation arising from clinical experience with PD patients that casts doubt on a strict interpretation of the direct/indirect pathway model. In the last few decades, a great effort has been undertaken to find a better treatment for PD than L-DOPA. This drug, however, remains the gold standard in the therapy of this neurodegenerative disease[88]. In fact, although long-term treatment with L-DOPA induces dyskinetic movements[62,89], it can be considered to be the most effective option in almost all phases of the disease. How does L-DOPA work? This drug is a precursor of endogenous DA that activates both D1-like and D2-like receptors. Although multiple mechanisms have been linked to this drug, only this dual pharmacological effect seems to provide the potent motor activation generated by L-DOPA in PD patients[90]. Selective D2/D3 receptor agonists are currently available and offer interesting therapeutic options[91], especially in the early phase of the disease. However, none of them are able to generate a therapeutic response similar to that achieved with L-DOPA. In fact, although it has been recently shown that D2 receptor activation can reduce motor disability in rodents, thereby reducing the risk of dyskinesia, high doses of these agonists might also interact with D1 receptors in producing both therapeutic and dyskinetic actions through heteromeric receptors[92]. More importantly, clinical therapeutic strategies, selectively activating the D1 receptors in PD, are neither feasible nor testable at present. Conversely, we also have to assume that it would be difficult, if not impossible, to observe the therapeutic effects of L-DOPA in PD patients in the absence of D2-like receptor stimulation. Accordingly, motor disabilities in PD patients treated with L-DOPA are markedly worsened by the use of classical neuroleptic agents (mainly antagonizing D2-like receptors) to control behavioral alterations[93]. Thus, we might conclude that the therapeutic efficacy of L-DOPA results from the activation of both D1-like and D2-like striatal receptors. Accordingly, it has been recently shown that experimental parkinsonism induced by MPTP leads to a decrease in dendritic spine density in both D1 and D2 receptor–containing MSNs and that intensive exercise leads to increased dendritic spine density and arborization in MSNs of both the pathways[94].

Oscillatory activity in PD: a link with direct/indirect pathways?

DA levels can rapidly modulate the synchronicity and oscillatory behavior of cortical and striatal circuits[95]. Moreover, electrophysiological studies in rodent and primate animal models of PD and in PD patients have discovered abnormally synchronized oscillatory activity at multiple levels of the basal ganglia–cortical loop[96,97]. This pathological synchronization correlates with akinesia and is suppressed by either dopaminergic therapies or DBS. In a rodent model of PD, it has been shown that striatal NMDA receptors gate cortico-pallidal synchronization[98], suggesting an interesting similarity with the NMDA-dependent forms of corticostriatal plasticity. Although the specific contribution of direct and indirect pathways to this oscillatory behavior remains to be established, one could speculate that the two pathways are abnormally coordinated in PD.

Conclusion

Although some recent findings raise possible doubts concerning a too rigid application of the direct/indirect pathway model, at present, the literature does not provide compelling evidence against it. However, we feel that this model, which tries to explain the entire complex activity of BG function in both physiological and pathological conditions, needs to be revised to integrate more recent scientific findings.

At this stage, it is possible to postulate a representation of the BG that, although not depicting all circuits, provides an interpretation of the 'filtering' function of the striatum in the BG activity as an integrative system of cortical glutamatergic and nigral dopaminergic inputs (**Fig. 4**). In this scenario, the direct and indirect pathways should not be seen as separate, parallel systems, as hypothesized in the classical interpretation of the model. On the contrary, as we describe here, the two pathways are structurally and functionally intertwined at least at two distinct levels: in the striatum, where the direct and indirect pathways communicate via the complex interneuronal network and the biochemical links between the two MSN subtypes, and outside of the striatum, where GPe collaterals may bridge the two pathways, potentially allowing the direct pathway to modulate the indirect pathway[52]. The existence of these bridging collaterals provides further support for the evidence obtained in non-human primates that striatal neurons projecting to either the GPi or the GPe show immunolabeling for both D1 and D2 DA receptors[56].

The interaction between the two pathways would therefore be dynamic in both physiological and pathological conditions, with DA and dopaminergic agents not being able to control a single pathway in isolation without influencing the other. In this context, the precise direction of striatal synaptic plastic changes would be driven by both the intensity of cortical/thalamic glutamatergic activation and the amount and the precise timing of DA release[59].

The coordinated action of the direct and indirect pathways during action initiation[44] and the concomitant activation of SNc make a circuit to signal start and stop of action sequences[99,100] (**Fig. 4**). Conversely, it is possible that, in PD, the alterations of the main forms of DA-dependent synaptic plasticity in MSNs could make the striatum unable to filter neuronal signals. Thus, the lack of coordinated activity between these two pathways will result in the inability of the PD patient to physiologically

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Box 1 Outstanding questions

- Do specific striatal MSNs express *in vivo* a single form of synaptic plasticity or can these neurons undergo different, and even opposite, forms of plasticity depending on their functional and metabolic state (membrane potential, energetic condition, endogenous striatal levels of DA)?
- Can striatal interneurons modulate the activity of MSN and, in turn, facilitate or inhibit motor activity depending on the form of synaptic plasticity expressed in a specific functional state?
- What are the specific physiological effects resulting from the activation of D1/D2 heteromers as well as of other DA heteromers?
- How do distinct levels of DA denervation, as observed during the evolution of the natural history of PD, differentially affect plasticity in direct/indirect pathway MSNs as well as in various subtypes of striatal interneurons?
- How is the activity of direct/indirect pathway MSNs altered by the pathological processes implicated in the different phases of nigral neurodegeneration in PD?
- Most animal investigations dealing with potential therapeutic interventions in the direct/indirect pathways have not been performed as blinded studies. Can similar results be obtained using a blind protocol as in many clinical trials?
- Will a selective activation of MSNs of either direct or indirect pathways in isolation and in conjunction be possible in the near future in humans?
- How would these specific activations affect the symptoms and the natural history of disabling neurodegenerative diseases of the BG such as PD and HD?

start and stop action sequences and in a global slowing of motor function, causing bradykinesia, freezing and gait festination (**Fig. 4**).

This interpretation integrates the classical direct/indirect hypothesis, as it considers the importance of striatal interneurons in striatal physiology and suggests that all MSNs might either facilitate or inhibit movement depending on the form of synaptic plasticity expressed in a certain moment. Future experimental and clinical studies will provide answers to the outstanding questions on the complex functions of the direct/indirect pathways (**Box 1**).

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ACKNOWLEDGMENTS

We thank A. Pisani for reading the manuscript and for critical discussion. This work was supported by Progetto di Ricerca di Interesse Nazionale (PRIN) 2011 2010AHHP5H (to P.C.) and Progetto del Ministero della Salute, Giovani Ricercatori (GR-2008-1142336 to B.P.; GR-2010-2316671 to V.G.).

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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