Bidirectional plasticity in striatonigral synapses: A switch to balance direct and indirect basal ganglia pathways

Jose J. Aceves,1,2 Pavel E. Rueda-Orozco,1,2 Ricardo Hernandez-Martinez,1 Elvira Galarraga,1 and Jose Bargas1,3

1Instituto de Fisiologia Celular-Neurociencias, Universidad Nacional Autonoma de México (UNAM), México City, D.F. Mexico 04510

There is no hypothesis to explain how direct and indirect basal ganglia (BG) pathways interact to reach a balance during the learning of motor procedures. Both pathways converge in the substantia nigra pars reticulata (SNr) carrying the result of striatal processing. Unfortunately, the mechanisms that regulate synaptic plasticity in striatonigral (direct pathway) synapses are not known. Here, we used electrophysiological techniques to describe dopamine D1-receptor-mediated facilitation in striatonigral synapses in the context of its interaction with glutamatergic inputs, probably coming from the subthalamic nucleus (STN) (indirect pathway) and describe a striatonigral cannabionoid-dependent long-term synaptic depression (LTD). It is shown that striatonigral afferents exhibit D1-receptor-mediated facilitation of synaptic transmission when NMDA receptors are inactive, a phenomenon that changes to cannabionoid-dependent LTD when NMDA receptors are active. This interaction makes SNr neurons become coincidence-detector switching ports: When inactive, NMDA receptors lead to a dopamine-dependent enhancement of direct pathway output, theoretically facilitating movement. When active, NMDA receptors result in LTD of the same synapses, thus decreasing movement. We propose that SNr neurons, working as logical gates, tune the motor system to establish a balance between both BG pathways, enabling the system to choose appropriate synergies for movement learning and postural support.

The basal ganglia (BG) control the learning and memory of procedures and habits, action selection, and the coordination and storage of motor programs (Cools 1984; Takakusaki et al. 2004; Grillner et al. 2005; Yin and Knowlton 2006; Graybiel 2008). The substantia nigra pars reticulata (SNr) and the internal segment of the globus pallidus (GPi) are the output nuclei of the BG receiving converging entries from both direct and indirect BG pathways (Alexander et al. 1986; Alexander and Crutcher 1990; Radnikow and Misgeld 1998; Smith et al. 1998; Ibanez-Sandoval et al. 2006; Deniau et al. 2007). Probably a choice of whether an action is executed or not occurs in these nuclei (Sanestani et al. 2011), since according to the “two pathways model” (Albin et al. 1989), direct pathway activation facilitates, whereas indirect pathway activation represses movements (Wichmann and DeLong 2003; Graybiel 2004; Grillner et al. 2005; DeLong and Wichmann 2007; Ibanez-Sandoval et al. 2007; Bateup et al. 2010; Kravitz et al. 2010). As in other systems, synaptic plasticity is a good candidate to comprise the mechanism that makes the selection (Kreitzer and Malenka 2007, 2008; Shen et al. 2008; Di Filippo et al. 2009). However, synaptic plasticity studies in the BG have been mostly restricted to the glutamatergic corticostriatal synapse (Calabresi et al. 2007; Kreitzer and Malenka 2008; Surmeier et al. 2009; Wickens 2009; Lovinger 2010). Nonetheless, it is well-known that GABAergic synapses also exhibit long-term synaptic plasticity (LTP) (Sjostrand et al. 2008; Castillo et al. 2011) and that they conform important connections along direct and indirect BG pathways (Guzman et al. 2003; Misgeld et al. 2007; Tecuapetla et al. 2007; Connolly et al. 2010; Tepper et al. 2010; Chuhma et al. 2011). Finally, LTD has been described on BG GABAergic synapses (Adermark et al. 2009; Rueda-Orozco et al. 2009). Therefore, a main goal of the present work is to initiate the study of LTD in an important GABAergic BG connection, such as the striatonigral synapse, a crucial endpoint of both direct and indirect BG pathways (Albin et al. 1989).

Another question concerns the role of dopamine in the choice between the direct and indirect pathways, perhaps interacting with synaptic plasticity (Surmeier et al. 2009; Wickens 2009). It is known that dopaminergic fibers and neurons innervate SNr (Misgeld 2004; Zhou et al. 2009; Rommelflanger and Wichmann 2010) and that they modulate its afferents: striatonigral (direct pathway) (Cameron and Williams 1993; Radnikow and Misgeld 1998; Aceves et al. 2011; Chuhma et al. 2011) and subthalamonigral (indirect pathway) (Ibanez-Sandoval et al. 2006) via presynaptic receptors (Yanovsky et al. 2003; Misgeld et al. 2007). Accordingly, here we study the dopaminergic modulation of striatonigral synapses and propose its possible role in the selection mechanism.

Synaptic plasticity and modulation, in particular LTD, have been proposed as cellular mechanisms to explain enduring changes of synaptic weights in microcircuits as well as learning and memory storage (e.g., Hebb 1949; Malenka and Bear 2004; Barbour et al. 2007; Misgeld et al. 2007). It is shown here that a form of striatonigral long-term depression (LTD) depends on presynaptic cannabinoid (CB1)-receptor activation and on the coincident activation of post-synaptic NMDA receptors. In contrast, presynaptic D1-receptor-mediated facilitation of striatognigral IPSCs (Radnikow and Misgeld 1998; Aceves et al. 2011; Chuhma et al. 2011) is elicited when NMDA-receptors are inactive (blocked). Consequently, the direct pathway synapses may
Synaptic plasticity in substantia nigra reticulata

Results

Synaptic facilitation or depression of striatonigral IPSCs depends on NMDA-receptor activation

The method to selectively evoke striatonigral IPSCs with field stimulation at the internal capsule while recording SNr neurons has been well documented (Radnikow and Misgeld 1998; Yanovsky et al. 2003; Beurrier et al. 2006; Misgeld et al. 2007; Rueda-Orozco et al. 2009; Connelly et al. 2010; Aceves et al. 2011). In brief, striatonigral and pallidonoigral IPSCs can easily be sorted out using several variables such as amplitude, short-term synaptic plasticity, and intensity-amplitude plots (Connelly et al. 2010; Aceves et al. 2011). Thus, although field stimulation may activate striatonigral or pallidonoigral terminals, we chose only the former for the present analysis. On the other hand, subthalamonoigral and other possible glutamatergic sources are completely or partially blocked during the following experiments. Time courses in Figure 1 illustrate striatonigral IPSCs before and after high-frequency stimulation (arrow HFS: two trains of stimuli at 100 Hz—see Material and Methods) (Mendoza et al. 2006; Rueda-Orozco et al. 2009) under two different conditions. The first condition includes both 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an AMPA/KA-receptor antagonist, plus 50 μM (2R)-amino-5-phosphonovaleric acid (APV), a NMDA-receptor antagonist (Fig. 1A; Ibañez-Sandoval et al. 2006; Rueda-Orozco et al. 2009). In this situation, IPSCs undergo a brief period of post-tetanic potentiation and thereafter maintain increased amplitude, as compared with the controls, for >20 min after HFS. The second condition suppressed APV from the bath saline so that internal capsule stimulation could also turn on subthalamonoigral synapses upon NMFA receptors (Ibañez-Sandoval et al. 2006; 2007; Aceves et al. 2011) in SNr neurons (Fig. 1B). In this alternative state, the outcome is the opposite: IPSCs decrease in their amplitude and maintain this depression for >20 min after a brief period of post-tetanic potentiation following HFS. Insets in Figure 1, A and B, show IPSCs evoked with paired stimuli and averaged along 10 min before (control left) or after HFS (the last 10 min: between 30 and 40 min as seen in the time courses). The time course in Figure 1C shows mean ± SEM of normalized IPSC amplitudes along time in the two conditions. In the presence of NMDA-receptor blockade, IPSCs amplitude remained ~200% above baseline >20 min after HFS (n = 7) (Fig. 1C, empty circles HFS + APV; P < 0.001). In contrast, unblocking NMDA receptors (absence of APV) lead to a long-lasting 40% depression in IPSCs amplitude as compared with baseline (n = 7) (Fig. 1C, filled circles APV; P < 0.03).

Figure 1. Striatonigral IPSC facilitation or depression depends on NMDA-receptor activation. (A) Time course of IPSC amplitude before and after high-frequency stimulation (HFS, arrow): ⋆ single responses; (•) the mean of 10 responses in this and similar frames. In this and similar figures, first IPSC is used to build the time course and second IPSC amplitude/first IPSC amplitude is the paired-pulse ratio (PPR). Mean amplitude and PPR are compared before and after HFS. Note, there is a brief period of post-tetanic potentiation, followed by a sustained facilitation in IPSC amplitude. CNQX and APV were present (inactive NMDA-R). (Top, inset) Paired responses before and after HFS. (B) Time course of an experiment similar to that shown in A, except that APV was absent from the superfusion (active NMDA-R): Post-tetanic potentiation evoked after HFS is now followed by a persistent IPSC depression. (Top, inset) Paired responses before and after HFS. (C) Normalized average ± SEM of time courses of IPSC amplitude with inactive (+APV; n = 7) and active (−APV; •, n = 7) NMDA receptors in two experimental samples. (D) Normalized average ± SEM of PPR with inactive (+APV, ⋆) and active (−APV, •) NMDA receptors in the same samples. (E,F) Box-density plots showing samples distributions. (*) P < 0.001; (**) P < 0.03.
Figure 1D shows that these changes in IPSC amplitude were accompanied by a decrease in the paired-pulse ratio (PPR) (empty circles; \( P < 0.03 \)) in the case of facilitation, and an increase in PPR (filled circles; \( P < 0.001 \)) in the case of depression, suggesting that both phenomena have a presynaptic contribution. Finally, box-density plots in Figure 1, E and F, illustrate the contrasting changes in IPSCs amplitude and PPR with (white boxes) and without APV (black boxes) in samples of experiments.

In summary, the only experimental difference to evoke either D1-receptor-mediated facilitation of synaptic transmission or synaptic depression in striatonigral synapses after HFS was the absence or presence of NMDA-receptor activation, respectively. Therefore, NMDA receptors are similar to switches that turn facilitation into depression in the same synapses.

Role of dopamine D1-class receptors on striatonigral synaptic facilitation

Experiments illustrated in Figure 2 intend to find the role of dopaminergic modulation in striatonigral facilitation and to see whether this is a form of long-term potentiation or not. It is known that a hallmark of striatonigral terminals is their presynaptic dopamine D1 receptors, which enhance IPSCs amplitude and thus direct pathway output (Radnikow and Misgeld 1998; Yanovsky et al. 2003; Aceves et al. 2011; Chuhma et al. 2011). Moreover, these presynaptic receptors appear to be continuously modulated by extracellular dopamine levels (Yanovsky et al. 2003; Aceves et al. 2011), which may be greatly enhanced by HFS for a long time (Cheramy et al. 1981; Reynolds et al. 2001; Wickens 2009).

First, it is shown that when 1 \( \mu \text{M} \) SCH-23390, a dopamine D1-receptor antagonist, is previously added to the bath saline, HFS is no longer capable of producing synaptic facilitation, but on the contrary, depression follows as a result (Fig. 2A), accompanied with a PPR increase (\( n = 9; \ P < 0.01 \); inset; Fig. 2B, filled circles), even if post-tetanic potentiation can still be evoked and NMDA receptors are blocked. In fact, striatonigral IPSC decreased in amplitude by the sole presence of SCH-23390 in all neurons of the sample (mean \( \pm \text{SEM} \)): 27 \( \pm \) 9% (Fig. 2B, empty circles; \( P < 0.01 \); bar indicates the presence of the antagonist; Yanovsky et al. 2003; Aceves et al. 2011). Synaptic depression after HFS in the presence of SCH-23390 was 60 \( \pm \) 19% (Fig. 1A,B, empty circles; \( P < 0.001 \); vs. control; \( P < 0.02 \); vs. SCH-23390 alone).

Therefore, a D1-receptor antagonist is enough to block synaptic facilitation and turn the result of HFS into a long-lasting synaptic depression.

The alternate experiment, that is, the use of 500 nM SKF-81297, a D1-receptor agonist, produced opposite actions: it facilitated striatonigral IPSCs by itself (Fig. 2C; Radnikow and Misgeld 1998; Aceves et al. 2011; Chuhma et al. 2011). D1-receptor activation evoked a significant IPSC increase with no signs of.
Striatonigral synaptic depression depends on endocannabinoids. (A) Striatonigral IPSC amplitude time course during addition of a cannabinergic CB1-receptor antagonist: 1 μM AM 251 (horizontal bar). The antagonist inhibited HFS-mediated depression (active NMDA-R). Note post-tetanic potentiation. (Top, inset) Paired responses before and after HFS. A small increase seen in some cases is nonsignificant. (B) Normalized and averaged IPSCs amplitude (○) and corresponding PPR (●) in a sample of experiments (n = 6; NS). Facilitation was nonsignificant. (C) Striatonigral IPSC amplitude time course during addition of a cannabinergic CB1-receptor agonist: 10 μM WIN 55212 (horizontal bar). Agonist depressed IPSC amplitude by itself with an increase of PPR (n = 6; P < 0.05). HFS evoked a more profound and long-lasting depression. (Top, inset) Paired responses before and after HFS. (D) Normalized and averaged IPSCs amplitude (○) and corresponding PPR (●) in a sample of experiments (n = 6; WIN: P < 0.05; WIN + HFS: P < 0.002). In a subsample of experiments (n = 4), 1 μM AM 251 (gray horizontal bar) was added once the depression was stable: the depression was not reversed, suggesting LTD.
intrinsic calcium is necessary to evoke striatonigral LTD. To test this hypothesis we used HFS while leaving NMDA receptors unblocked, a maneuver that evokes LTD (see Fig. 1B,C). In contrast, a sample of experiments we tested this protocol in the presence of the calcium chelator, 20 μM BAPTA, in the intracellular medium (recording pipette): Chelation of intracellular Ca²⁺ in the recorded SNr neuron impeded HFS-induced LTD (Fig. 4A). No sign of a PPR change was seen in this situation, indicating that presynaptic modulation was impeded by a postsynaptic maneuver (Fig. 4A, right panels). Synaptic plasticity in substantia nigra reticulata. www.learnmem.org 768 Learning & Memory

Figure 3C shows that a CB₁-receptors agonist, 10 μM WIN-55212, decreases IPSCs amplitude by itself (Fig. 3D, empty circles) by 61 ± 10% (n = 6; P < 0.05) (Szabo et al. 2000; Wallmichrath and Szabo 2002; Yanovsky et al. 2003; Misgeld et al. 2007; Romo-Parra et al. 2009). This action is reversible and is accompanied by a decrease in PPR of 154 ± 21% (Fig. 3D, filled circles; n = 6; P < 0.05), supporting a presynaptic contribution. When HFS is applied during WIN-55212, a further, stronger, and longer-lasting decrease in IPSCs amplitude is obtained (Fig. 3C,D, empty circles): 37 ± 19% (P < 0.002) even if compared with IPSCs during WIN-55212 alone (P < 0.05). In a sample of experiments (n = 4) we tested the cannabinoergic antagonist, 1 μM AM-251, to see whether it could reverse the long-lasting depression induced by HFS once it was stable. It was observed that AM-251 could not reverse the already installed depression (Fig. 3D, gray bar), suggesting, as the most parsimonious working hypothesis, that this HFS-induced depression is a true long-term depression (LTD) (e.g., Mulkey et al. 1994; Gerdeman and Loring 2003; Hashimoto-dani et al. 2005; Massey and Bashir 2007; Sergeeva et al. 2007; Szabadits et al. 2011; Xue et al. 2011) in need of further exploration. Because once-established time courses with and without AM-251 were similar, all data were pooled together in the time courses illustrated in Figure 3D.

To summarize, similar to corticostriatal and corticoacumbinal glutamatergic synapses (Gerdeman et al. 2002; Robbe et al. 2002; Kreitzer and Malenka 2007; Shen et al. 2008), as well as hippocampal (Chevaleyre and Castillo 2003; Ohno-Shosaku et al. 2007) and cerebellar LTD (Safo and Regehr 2005), putative striatonigral LTD depends on cannabinoergic CB₁ receptors.

An increase in postsynaptic intracellular calcium is necessary to evoke striatonigral LTD

How to explain NMDA-receptor dependency of HFS-induced LTD? One hypothesis is that increases in intracellular Ca²⁺ accompanying NMDA-receptor activation in SNr neurons (Ibañez-Sandoval et al. 2006, 2007) augments the synthesis of endogenous cannabinoids (e.g., Gerdeman et al. 2002; Robbe et al. 2002; Chevaleyre and Castillo 2003; Massey and Bashir 2007; Ohno-Shosaku et al. 2007), which, in turn, act as retrograde messengers that target presynaptic striatonigral terminals (Yanovsky et al. 2003). To test this hypothesis we used HFS while leaving NMDA receptors unblocked, a maneuver that evokes LTD (see Fig. 1B,C). In contrast, a sample of experiments we tested this protocol in the presence of the calcium chelator, 20 μM BAPTA, in the intracellular medium (recording pipette): Chelation of intracellular Ca²⁺ in the recorded SNr neuron impeded HFS-induced LTD (Fig. 4A). No sign of a PPR change was seen in this situation, indicating that presynaptic modulation was impeded by a postsynaptic maneuver (Fig. 4A, right panels). Still, we checked for NMDA-receptors contribution in SNr neurons during HFS. Using current-clamp recordings at a membrane potential similar to the holding potential used for voltage-clamp recordings, i.e., −80 mV, as well as high-chloride concentrations (see Materials and Methods) to evoke depolarizing GABAergic synaptic events, HFS was applied either in the presence of both, CNQX + APV, to avoid both activation of AMPA/KA and NMDA receptors, or in the presence of CNQX alone to avoid activation of AMPA/KA receptors only. The aim was to compare the HFS responses in both conditions to see whether we could identify a NMDA-receptor contribution to the response, and then confirm it as a potential source of intracellular calcium. The results are illustrated in Figure 4, B and C. Postsynaptic responses in each condition were clearly different (Fig. 4B). The subtraction of these responses yielded the NMDA-receptor contribution to the postsynaptic response (Fig. 4C): a slow depolarization >10 mV in amplitude and lasting more than a second, most of it posterior to the train of responses given by HFS (black bar in Fig. 4B,C; n = 6; P < 0.02; black trace = mean; gray surround = SEM). Less-negative membrane potentials would only enhance this component (Ibañez-Sandoval et al. 2006). Clearly, NMDA-receptor activation during HFS may be a source of intracellular calcium for cannabinoiod synthesis (Ohno-Shosaku et al. 2007). Therefore, potential conditions are met to explain why NMDA-receptors activity may induce cannabinoiod synthesis and IPSC depression (Yanovsky et al. 2003). This demonstration does not exclude other receptor (Surmeier et al. 2009) contributions. Thus, chelation of
intracellular calcium inhibited cannabinoid synthesis, impeding IPSC depression (Figs. 3A, 4A). However, despite the fact that CB1-receptor blockade and intracellular calcium chelation blocked IPSC depression, neither one induced IPSC facilitation after HFS. To see whether this result was due to cannabinoid syn-
thesis in neighboring SNr neurons, we repeated the experiment after HFS. To see whether this result was due to cannabinoid syn-the block IPSC depression, neither one induced IPSC facilitation (Kriite et al. 1999), the action of D2-dopamine receptors on dopamine neurons (Giuffrida et al. 1999), or subsequent signaling cascades or receptors set into action after NMDA-receptors activation (Mukley et al. 1994; Hashimoto et al. 2005; Massey and Bashir 2007; Sergeeva et al. 2007; Szabadits et al. 2011; Xue et al. 2011). These alternative routes are left for future investigation and are out of the scope of the present report; even for the corticostriatal synapse, some of these signaling pathways are still under debate (Surmeier et al. 2009; Wieckens 2009). In addition, the need of NMDA-receptors activation does not completely exclude long-lasting depression due to CB1-receptor activation only (e.g., Kriite and Malenka 2005).

CB1 signaling prevails upon D1 signaling

Because both phenomena facilitation and depression are triggered by the activation of presynaptic receptors, D1 and CB1, respec-
tively, we asked whether any kind of HFS-induced plasticity is left when both receptors are blocked. When both receptor antag-onists, SCH-23390 and AM-251, are administered together, no change in IPSC amplitude or PPR could be evoked by HFS (Fig. 5A). Nevertheless, post-tetanic potentiation could still be evoked. Notwithstanding, this experiment does not discard other kinds of plasticity under different stimulation paradigms, it only supports that opposite dopaminergic and cannabinergic influ-
ences may act as push–pull regulators of GABA release in striatoni-gral connections (Yanovsky et al. 2003).

However, cannabinergic LTD may need the indirect pathway (STN afferents on SNr to activate NMDA receptors), while dopami-
nergic facilitation belongs to the direct pathway. Therefore, in order to observe whether these pathways interact at this level, it is necessary to know which of the two actions prevails when both receptors are turned on. Figure 5B illustrates what happens when dopaminergic facilitation is turned on before cannabiner-
nergic-induced depression. This was achieved by adding the D1-receptor agonist, 1 μM SKF-81297, to the superfusion and by applying additional HFS. These maneuvers evoked a D1-receptor-mediated facilitation (Figs. 1A, 2C,D, 5B). When this facilitation reached a steady-state after several minutes, a cannabinergic CB1-receptor agonist, 10 μM WIN-55212, was added to the bath saline. Facilitation turned into depression almost immediately (Fig. 5B, empty circles, n = 8; P < 0.002) with a concomitant change in PPR (Fig. 5B, filled circles; P < 0.05), again suggesting that the facilitation is not a form of LTP and that CB1-mediated actions can overcome D1-mediated actions. The next experiment (Fig. 5C) shows that the action of CB1-receptor agonists is specific because it can be blocked by the selective antagonist: 1 μM AM-251 (Fig. 5C). Therefore, a cannabinoid antagonist may pro-
long the enhancement of direct pathway activation and thus pro-
mote movement (Kravitz et al. 2010).

To conclude, striatlonigral terminals are subject to two main antagonistic and presynaptically driven actions: one is the D1-receptor-dependent facilitation of GABA release and other is the CB1-receptor-dependent decrease in IPSC amplitude (Yanovsky et al. 2003), the later perhaps becoming a truly LTD as described in other synapses (Gerden et al. 2002; Robbe et al. 2002; Chevaleyre et al. 2003; Kriite and Malenka 2005, 2007; Szafro and Regehr 2005; Mendoza et al. 2006; Ohno-Shosaku et al. 2007; Shen et al. 2008). Activity in each one of these afferents may arrive simultaneously or in an alternating way as discussed below.

Discussion

Previous experimental evidence supports that SNr integrates information coming from both direct and indirect pathways of

Figure 5. Cannabinergic modulation overcomes dopaminergic one. (A) Normalized and averaged IPSCs amplitude (RT) and corresponding PPR (RT) in a sample of experiments (n = 7) in which both dopamine D1-receptor antagonist: 1 μM SCH-23390 and cannabinergic CB1-receptor antagonist: 1 μM AM-251 were added to the bath saline (inactive NMDA-R). Both HFS-mediated facilitation and depression were abolished. (Top inset) Paired responses before and after HFS in a representative experiment. (B) Normalized and averaged IPSCs amplitude (RT) and corresponding PPR (RT) in a sample of experiments (n = 8) that exhibit D1-receptor-mediated facilitation (500 nM SKF-81297). HFS was also given 10 min after facilitation was stable. In these conditions, the CB1-receptor agonist, 10 μM WIN-55212, was capable of changing facilitation into depression, confirming that cannabinoids can reverse dopaminergic actions even if NMDA receptors are inactive. (Top, inset) Paired responses before and after HFS in a representative experiment. (C) Normalized and averaged IPSCs amplitude (RT) and corresponding PPR (RT) in a sample of experiments (n = 8) that exhibit D1-receptor-mediated facilitation (500 nM SKF-81297) in the presence of the CB1-receptor antagonist: 1 μM AM-251. HFS was also given 10 min after facilitation was stable. In these conditions, the CB1-receptor agonist, 10 μM WIN-55212, was no longer capable of turning facilitation into depression, confirming that cannabinergic presynaptic actions are specific. (Top, inset) Paired responses before and after HFS in a representative experiment.
the BG (Nakanishi et al. 1987; Bevan et al. 1994; Maurice et al. 1999; Deniau et al. 2007). In our hands, recording synaptic events from either of these synapses: striatonigral (Aceves et al. 2011) or subthalamonigral (Ibáñez-Sandoval et al. 2006) always elicited synaptic events in SNr postsynaptic neurons. How SNr neurons integrate, balance, and choose between these inputs is mostly unknown. Here, we set forth an initial working hypothesis of such a mechanism (Fig. 6): First, it was shown that D1-mediated facilitation or CB1-mediated long-lasting depression can be expressed by the same striatonigral (direct pathway) synapses onto SNr neurons (Yanovsky et al. 2003). Facilitation (Fig. 6A) depends on dopamine D1-receptor activation of striatonigral synapses (Florán et al. 1990; Radnikow and Misgeld 1998; Aceves et al. 2011; Chuhma et al. 2011), which can be reliable and preferentially stimulated in parasagittal slices (Radnikow and Misgeld 1998; Beurrier et al. 2006; Connelly et al. 2010; Aceves et al. 2011). This facilitation can be evoked by HFS and may last for several minutes (e.g., Reynolds et al. 2001; Wickens 2009), depending on inactive NMDA receptors and endogenous extracellular dopamine levels. However, once evoked and stable, facilitation can be rapidly turned off by D1-receptor antagonists or CB1-receptor agonists. Thus, it is suggested that it does not constitute a true LTP (Kreitzer and Malenka 2008).

Although SNr neurons express D1 class receptors postsynaptically (Zhou et al. 2009), cells were voltage clamped at −80 mV and filled with intracellular solutions containing Cs+ and QX-314, making it very hard to stimulate modulatable voltage-dependent currents. In addition, plastic changes reported here were accompanied by corresponding changes in the paired-pulse responses and not accompanied by changes in the input resistance of postsynaptic neurons. Therefore, D1-receptor-dependent IPSC facilitation can be explained by a predominant striatonigral afferent modulation (Fig. 6; Radnikow and Misgeld 1998; Aceves et al. 2011; Chuhma et al. 2011). Nonetheless, a possible role of the signaling cascade associated with postsynaptic D1-class (D1-type) receptors in SNr neurons is in need of future investigation (Zhou et al. 2009).

A second element of the gating mechanism is a long-lasting depression depending on cannabinergic CB1-receptor activation (Fig. 6B). Depression of striatonigral output depends on active NMDA receptors (Ohno-Shosaku et al. 2007). Induction of cannabinergic CB1-receptor-dependent HFS-mediated depression was blocked by intracellular Ca2+ chelation and by CB1-receptor antagonists, even when NMDA receptors were active, suggesting that its manifestation is triggered by endogenous cannabinoid release (Gerdeeman et al. 2002; Robbe et al. 2002; Chevaleyre et al. 2003; Yanovsky et al. 2003; Safo and Regehr 2005; Kreitzer and Malenka 2005, 2007, 2008; Massey and Bashir 2007; Ohno-Shosaku et al. 2007; Shen et al. 2008). Plastic changes are accompanied by corresponding increases in paired response ratios that mirror those produced by dopaminergic facilitation. But, in contrast to D1-receptor HFS-mediated facilitation, CB1-receptor-mediated depression cannot be reversed by receptor antagonists once it has reached a steady-state for several minutes, suggesting the establishment of a true LTD in need of further attention (Gerdeeman et al. 2002; Robbe et al. 2002; Chevaleyre et al. 2003; Gerdeeman and Lovinger 2003; Kreitzer and Malenka 2005, 2007, 2008; Massey and Bashir 2007; Shen et al. 2008; Surmeier et al. 2009; Lovinger 2010). Finally, substantial evidence supporting presynaptic modulation of nigral afferents by both D1 and CB1 receptors (Szabo et al. 2000; Yanovsky et al. 2003; Misgeld et al. 2007; Aceves et al. 2011) makes infeasible that long-term plastic changes may be mediated, modulated, or triggered by these receptors and their associated signaling. Possible mechanisms associated with cannabinergic actions have to be investigated in future studies (e.g., Mulkey et al. 1994; Iribe et al. 1999; Massey and Bashir 2007; Szabadits et al. 2011; Xue et al. 2011).

**SNr neurons as coincidence detectors switching gates**

In summary, expression of either facilitation or depression depends on NMDA-receptor activation of SNr neurons during synaptic activity: When NMDA-receptors are active, the end result is depression of striatonigral synapses. This outcome would repress movement execution (Kravitz et al. 2010). When NMDA receptors are inactive, the result is D1-dependent facilitation of striatonigral synapses. This outcome would promote movement execution (Kravitz et al. 2010). These hypotheses could be tested in vivo. Note that a linking element between direct and indirect BG pathways is the activity of NMDA receptors, because they can be set into action by subthalamonigral inputs (Beurrier et al. 2006; Ibáñez-Sandoval et al. 2006, 2007), the last step of the indirect BG pathway, or by brain-stem ascending inputs (Takakusaki et al. 2003, 2004; Surmeier et al. 2011). Thus, the indirect pathway can have an influence on the actions of the direct pathway via the activation of postsynaptic NMDA receptors. This finding sets the basis of a switching mechanism between both pathways (Fig. 6; Table 1). That being said, other interactions and receptors are not being discarded, as those from brain stem.

BG output nuclei may “gate” the execution of different classes of motor procedures, from central pattern-generated movements (Grillner et al. 2005) to acquired complex habits.
Slice preparation

The experiments were performed on brain slices (300-μm thick) obtained from male Wistar rats (PD20-40). Animals were anesthetized, intracardially perfused, and decapitated. The brain was quickly removed, cut using a vibrating microtome (Ted Pella) and immersed in a 4°C solution with the following composition (in millimolars): 126 choline-Cl, 2.5 KCl, 1 MgCl₂, 2 CaCl₂, 26 NaHCO₃, 10 glucose (pH = 7.4, saturated with 95% O₂ and 5% CO₂, 298 mOsm/L). Slices were transferred to a saline solution containing (in millimolars): 123 NaCl, 2.5 KCl, 1.3 MgCl₂, 2.0 CaCl₂, 26 NaHCO₃, 10 glucose (pH = 7.4, 25–27°C saturated with 95% O₂ and 5% CO₂, 298 mOsm/L). The slices were left for equilibration in the oxygenated saline at room temperature for ≥ 1 h.

Electrophysiology

After equilibration, single slices were transferred to a recording chamber and superfused continuously with oxygenated saline (4–5 mL/min). Whole-cell recordings were performed using infrared differential interference contrast (IR-DIC) microscopy with an upright microscope and a digital camera. IPSCs were recorded with the help of an amplifier (Dagan Corp.). Data acquisition was performed with an AT-MIO-16E4 board (National Instruments) and software designed in the LabView environment. Patch micropipettes (2–6 MΩ) were pulled (Sutter Instrument) from borosilicate glass tubes (1.5 mm OD, WPI) and were filled with internal saline containing (in millimolars): 72 K₂HPO₄, 36 KCl, 1.1 EGTA, 10 HEPS, 2 CaCl₂, 1 MgCl₂, 2 ATP-Mg, 0.3 GTP-Na, 5 QX-314 (pH 7.3 and 272–275 mOsm/L); in some experiments 20 mM of BAPTA were added. High-chloride internal solution was used to obtain IPSCs as inward currents or IPSPs as depolarizing potentials at a membrane potential of ~ 80 mV.

IPSCs were evoked with field stimulation via sharp (pencil shape) concentric bipolar electrodes (12 μm at the tip; 10 kΩ; FHC) attached to an isolation unit (Digimiter). Stimulation electrodes were positioned at the internal capsule near the SNr in sagittal brain slices (Acese et al. 2011). Distance between the recording and stimulation electrodes were 0.5–1.0 mm in all configurations. All recordings were done in the presence of the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate/kainate receptor (AMPA/KA) receptor antagonist, (CNQX, 10 μM) and, when stated, in the presence of N-methyl-D-aspartate (NMDA) antagonist (2R)-α-amino-5-phosphonovaleric acid (APV, 50 μM).

To obtain baseline-paired inhibitory postsynaptic currents (IPSCs), two shocks with a 50-msec interpulse interval were delivered at a rate of 0.1 Hz. Stimulation parameters were adjusted to obtain currents ranging from 100 to 500 pA during the control. The paired-pulse ratio (PPR) was expressed as the amplitude ratio IPSC₂/IPSC₁. To induce long-term synaptic plasticity a high-frequency stimulation (HFS) paradigm was used: two trains of 100 Hz (1 sec each, total of 200 stimuli) were delivered with an inter-train interval of 10 sec (Mendoza et al. 2006). To induce short-term synaptic plasticity, trains of 10 stimuli were delivered at 10 Hz every 10 sec. These stimuli helped to select striatonigral inputs from pallidonoigral inputs (Connelly et al. 2010; Acese et al. 2011).

All drugs were stored in stock solutions to be dissolved in their final concentrations into the superfusion saline. 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline disodium salt, CNQX, an α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate/kainate (AMPA/KA) receptor antagonist, (2R)-α-amino-5-phosphonovaleric acid, APV, (±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide, SKF 81297, and R(+)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride, SCH 23950, were obtained from Sigma-Alrich-RBI. 1,2-bis(o-aminoophenoxyl)ethane-N,N',N",N"-tetraacetic Acid, 4Na, BAPTA, was obtained from Calbiochem (Darmstadt, Germany), (R)-(+)-(2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1-naphthalenylmethyl ane mesylate, WIN 55212, and N(piperidin-1-yl)-5-(4-isodo- phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, AM 251, were obtained from Tocris.

Data analysis

Given the size and nature of our data, free-distribution statistics were used to compare the samples: Wilcoxon’s t-test and Friedman repeated-measures analysis of variance on ranks and Student-Newman-Keuls tests were used when several changes were done on the same sample. Significance was fixed at 0.05.

Acknowledgments

We thank A. Laville and D. Tapia for technical support and advice. This work was supported by a grant from Programa de Investigación Multidisciplinaria de Proyectos Universitarios de Liderazgo y Superación Académica (IMPULSA)-Universidad Nacional Autónoma de México (UNAM) to J.B. and E.G.; Dirección General de Asuntos del Personal Académico (DGAPA)-UNAM Grants IN-205610 to J.B. and IN-206010 to E.G.; the Miguel Alemán A. C. Foundation; and the Mexico-Germany Agreement Consejo Nacional de Ciencia y Tecnología-Deutsche Forschungsgemeinschaft (CONACyT-DFG) grant J0110/193/10 FON.INST.-29-10 to J.B. and CONACYT grants 98004 to E.G. and 154131 to J.B. P.E.R.-O. was a UNAM postdoctoral fellow. J.A. and R.H.-M. are students at the Biomedicine graduate school (UNAM) and receive or received CONACYT (Mexico) fellowships.

References


Received July 12, 2011; accepted in revised form October 4, 2011.