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Toward a Model of Retrograde Regulation of Striatal Synaptic Depression

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Review of Tozzi et al.

As the primary input nucleus of the basal ganglia, the striatum receives considerable glutamatergic information from cortical and thalamic neurons. Most striatal neurons (~95%) are GABAergic medium-sized spiny neurons (MSNs), which can be subdivided into two populations according to their projection targets and their selective expression of dopamine receptor types. Striatonigral MSNs express D₁-type receptors (D₁Rs) and constitute the direct pathway of the basal ganglia, whereas striatopallidal MSNs express D₂-type receptors (D₂Rs) and constitute the indirect pathway.

An important form of plasticity commonly observed in striatal MSNs, regardless of their projection targets and dopamine receptor types expressed, is a long-lasting reduction of synaptic strength in their excitatory glutamatergic inputs [i.e., long-term depression (LTD)] (Calabresi et al., 1992). LTD in MSNs appears to be initiated by postsynaptic endocannabinoid (eCB) synthesis, which acts as a retrograde signal to reduce glutamate release by activating presynaptic CB₁ receptors (CB₁Rs). eCB-mediated striatal LTD is strongly dependent upon D₂R stimulation, as well as

group I metabotropic glutamate receptors (mGluR_{1/5}) and L-type Ca²⁺ channels (for review, see Di Filippo et al., 2009). This leaves an important question unanswered: how does a postsynaptically initiated, D₂R-dependent phenomenon induce LTD in all MSNs if D₂Rs are expressed in only a subset of these neurons? Recent studies have attempted to address this question (Wang et al., 2006; Kreitzer and Malenka, 2007; Tozzi et al., 2011).

Wang et al. (2006) suggested that the relevant D₂Rs mediating striatal LTD were those expressed in cholinergic interneurons that, despite their low abundance (<5%), project to virtually all MSNs. In their proposed model, stimulation of D₂Rs in these neurons decreases their firing rate, reducing both acetylcholine (ACh) release and M1-type muscarinic ACh receptor (M₁R) stimulation. Postsynaptic M₁Rs are located near glutamatergic synapses in both direct and indirect pathway MSNs and they inhibit L-type Ca²⁺ channels (Olson et al., 2005). Therefore, transient interruption of the tonic ACh-mediated M₁R stimulation would be expected to lead to increased intracellular Ca²⁺ concentration and local eCB release in MSNs, which in turn would stimulate presynaptic CB₁Rs reducing glutamatergic drive in synapses onto both striatonigral and striatopallidal MSN populations.

Contrary to this view, Kreitzer and Malenka (2007) reported that glutamatergic synapses onto D₂R-expressing MSNs were more excitable than those formed onto D₁R-expressing MSNs, and that D₂R

stimulation led to eCB-mediated LTD exclusively in striatopallidal neurons, highlighting the role of postsynaptic D₂Rs in striatal LTD. Selective striatopallidal eCB-LTD was also found after mGluR_{1/5} stimulation, which led Kreitzer and Malenka (2007) to conclude that eCB-mediated LTD was more likely to occur in indirect pathway neurons.

In a recent paper published in *The Journal of Neuroscience*, Tozzi et al. (2011) provide a model of D₂R-dependent eCB-mediated LTD that could potentially reconcile the aforementioned studies. The main feature of the Tozzi et al. (2011) model is the involvement of the adenosine A_{2A} receptors (A_{2A}Rs), which are highly enriched in the striatum and tightly counteract D₂Rs at multiple levels (Schiffmann et al., 2007). Importantly, A_{2A}Rs are not only selectively expressed in D₂R-expressing striatopallidal MSNs, but are also expressed presynaptically on corticostriatal and thalamostriatal terminals, where they are thought to modulate glutamate release (Schiffmann et al., 2007; Ferré et al., 2010). Although Tozzi et al. (2011) also examined responses in dopamine-depleted striatal slices (a model of Parkinson's disease), here we will focus on their findings in physiological conditions.

In the first set of experiments, Tozzi et al. (2011) studied the involvement of D₂Rs and A_{2A}Rs in eCB-mediated synaptic depression in *ex vivo* striatal rat slices (Tozzi et al., 2011, their Fig. 1). They performed both intracellular and whole-cell patch-clamp recordings from MSNs of the dorsal striatum.

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Figure 1. Model of striatal plasticity induced in glutamatergic synapses onto both striatonigral and striatopallidal MSN populations in response to concomitant D_2 and A_{2A} receptor modulation. Coadministration of D_2 receptor agonist [quinpirole (Quin)] and A_{2A} receptor antagonist (ZM or ST) can induce sustained reduction of glutamate release in striatonigral and striatopallidal MSNs through at least three different sequences of events (white, blue, and gray numbered pathways). Postsynaptic modulation of D_2/A_{2A} receptors in striatopallidal neurons may reduce glutamate release ($\downarrow$ Glut) through local retrograde endocannabinoid signal (white pathway, 1–3). In parallel, blockade of presynaptic A_{2A} receptors located in glutamatergic terminals onto cholinergic interneurons, plus stimulation of D_2 receptors expressed in these neurons, may interrupt their firing, ultimately leading to decreased acetylcholine release ($\downarrow$ ACh, blue pathway, 1–3). The reduced stimulation of M_1 receptors may remove the inhibition exerted on L-type Ca^{2+} channels, promoting calcium influx, endocannabinoid production, and reduced glutamate release onto both populations of MSNs (blue pathways 4–6). Finally, blocking presynaptic A_{2A} receptors located in glutamatergic terminals onto striatonigral MSNs may additionally reduce the probability of release at these synapses (gray pathway, 1 and 2). Note that, under this scheme, glutamatergic synapses onto both striatonigral and striatopallidal neurons receive at least two different signals that reduce release, in agreement with the equivalent decrease of glutamatergic transmission found by Tozzi et al. (2011) in both MSN populations.

Single 0.1 Hz stimulations of corticostriatal fibers (in the presence of GABA_A receptor antagonist bicuculline) were used to induce EPSPs and EPSCs. Once stable recordings were established, the D_2 agonist quinpirole was applied either alone or in combination with two different A_{2A} antagonists [ZM24138 (ZM) or ST1535 (ST)]. Long-lasting depression of EPSP and EPSC amplitudes, reflecting a decrease in striatal glutamatergic transmission, was produced only when D_2 agonist and an A_{2A} antagonist were coapplied. Importantly, LTD responses were largely pre-

vented by the CB_1 R antagonist AM251, pointing to eCB involvement in this process (Fig. 1).

In view of the controversy reviewed above, the authors sought to determine whether D_2 R/ A_{2A} R modulation differentially affected synaptic activity in terminals synapsing onto either D_2 -striatopallidal or D_1 -striatonigral neurons (Tozzi et al., 2011, their Fig. 2). As with the aforementioned studies, Tozzi et al. (2011) took advantage of bacterial artificial chromosome (BAC) transgenic mice, a tool that allows an accurate identification of neuronal popula-

tions *in situ* (Gong et al., 2003). These mice hold a BAC transgene in which EGFP expression is regulated by D_1 receptor (D_1 -EGFP line) or D_2 receptor (D_2 -EGFP line) promoter sequences, offering a clear visualization of D_1 -striatonigral and D_2 -striatopallidal MSNs, respectively. In these mice, equivalent reductions of EPSC amplitude were observed in D_1 -EGFP and D_2 -EGFP MSNs when quinpirole and ZM were coapplied. Furthermore, the same proportion ($\sim 70\%$) of D_1 - and D_2 -EGFP neurons showed depressed glutamatergic transmission. These results indicate that concomitant modulation of D_2 and A_{2A} receptors influences plasticity to the same extent in both MSN populations (Fig. 1), in direct contrast to the observations of Kreitzer and Malenka (2007).

To further elucidate the involvement of the eCB system and to confirm a change in presynaptic release, the authors next performed a series of paired-pulse electrophysiology experiments in *ex vivo* striatal rat slices (Tozzi et al., 2011, their Fig. 3). When quinpirole and ZM or ST were coapplied, the EPSC paired-pulse ratio increased, suggesting a decrease of glutamate release probability. Importantly, CB_1 R antagonist AM251 blocked this effect, pointing to a direct eCB/ CB_1 R involvement in this process. The role of presynaptic CB_1 R was further confirmed through occlusion experiments, in which the CB_1 R agonist WIN reduced EPSC amplitude by itself, an effect that was not strengthened by co-application of quinpirole and ZM.

Finally, Tozzi et al. (2011) performed Ca^{2+} buffering studies to explore a possible link between the pharmacological effects of D_2 R/ A_{2A} R and eCB retrograde diffusion. Interestingly, inclusion of BAPTA (a Ca^{2+} chelating agent) in the patch pipette completely prevented the synaptic depression induced by quinpirole plus ZM. Indeed, although not discussed by the authors, quinpirole plus ZM application appeared to initially enhance EPSC amplitude (Tozzi et al., their Fig. 3C). Importantly, intracellular BAPTA did not prevent the intrinsic effects of the CB_1 R and GABA_BR agonists WIN and baclofen on EPSC amplitude. Altogether, these results point to the eCB system as the key mediator of the reduction of synaptic glutamate transmission on presynaptic terminals. As illustrated in Figure 1, this process requires a postsynaptic increase of intracellular Ca^{2+} in MSNs promoted by D_2 R and A_{2A} R modulation (Fig. 1, white numbered pathway).

As mentioned above, previous studies have described an involvement of cholinergic interneurons in D_2 -dependent and

eCB-mediated LTD in both striatonigral and striatopallidal MSNs (Wang et al., 2006). To explore whether their observed effect was related to acetylcholinergic neurotransmission, Tozzi et al. (2011) performed whole-cell recordings on cholinergic interneurons and showed that the combination of low-dose quinpirole and ZM reduced the interneurons' tonic firing rate (Tozzi et al., 2011, their Fig. 7). Further, the authors demonstrated that inhibition of M_1 Rs by pirenzepine prevented EPSC amplitude reduction in MSNs by quinpirole and ZM, an effect that was again rescued by WIN (Tozzi et al., 2011, Fig. 8). In agreement with previous reports (Wang et al., 2006), these findings implicate cholinergic interneurons in the propagation of D_2 R-dependent synaptic plasticity to all MSN populations and highlight the role of A_{2A} R in this process. This mechanism involves postsynaptic M_1 Rs, which, through L-type Ca^{2+} channels, may ultimately lead to eCB formation and retrograde signaling (Fig. 1, blue numbered pathway).

In building a model encompassing their findings, Tozzi et al., 2011, hypothesized that D_2 R and A_{2A} R are coexpressed on striatal cholinergic interneurons, a possibility that was confirmed by triple immunofluorescence studies, in which D_2 R and A_{2A} R were colabeled in combination with a marker for cholinergic interneurons (Tozzi et al., 2011, their Fig. 6). However, these results should be considered carefully, since D_2 R and A_{2A} R were detected with primary antibodies from the same host, a delicate method that relies on the monovalency of the F_{ab} fragment to prevent cross-reactivity, but that may produce interference if not saturated properly (Owen et al., 2010). Despite D_2 R expression in striatal cholinergic interneurons being quite clear, A_{2A} R expression in these neurons is not. In fact, the restricted expression of A_{2A} R exclusively in enkephalin-positive striatopallidal MSNs has been convincingly demonstrated (Schiffmann et al., 2007; Ferré et al., 2010) and strong evidence against its expression in

cholinergic interneurons has recently been provided (Durieux et al., 2009). In the latter work, the authors genetically ablated all striatal neurons that express the *adora2a* (A_{2A} R) gene, clearly showing that only striatopallidal neurons degenerate, whereas all types of interneurons, including cholinergic interneurons, remain unaltered (Durieux et al., 2009, their supplemental Fig. 4).

Nevertheless, it is possible to reconcile Tozzi et al.'s (2011) results, and probably those obtained in the previous studies, by considering the presynaptic distribution of A_{2A} Rs in glutamatergic terminals, in addition to their postsynaptic expression in D_2 -striatopallidal MSNs (Fig. 1, blue and gray numbered pathways). A_{2A} Rs may indeed follow a defined pattern of presynaptic distribution and so modulate glutamate release in direct-pathway corticostriatonigral and thalamostriatal terminals (Schiffmann et al., 2007; Ferré et al., 2010). In line with this view, thalamostriatal terminals have recently been shown to regulate cholinergic interneuron bursting activity in response to salient stimuli, and this modulates the gating of corticostriatal activity and the selective recruitment of D_2 -striatopallidal MSNs to drive attentional shift (Ding et al., 2010).

All together, emerging studies are clarifying the difficult problem of how a signal initiated in a subset of striatal neurons is able to propagate to all MSNs. As outlined by Tozzi et al. (2011), this complex regulation likely involves almost all known neuromodulatory systems operating in the striatum, from the primary dopamine signal to local eCB-mediated retrograde transmission, going through more tonically propagating signals such as the adenosinergic and acetylcholinergic systems.

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