Dual Dopaminergic Regulation of Corticostriatal Plasticity by Cholinergic Interneurons and Indirect Pathway Medium Spiny Neurons

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SUMMARY

Endocannabinoid (eCB)-mediated long-term depression (LTD) requires dopamine (DA) D2 receptors (D2Rs) for eCB mobilization. The cellular locus of the D2Rs involved in LTD induction remains highly debated. We directly examined the role in LTD induction of D2Rs expressed by striatal cholinergic interneurons (Chls) and indirect pathway medium spiny neurons (iMSNs) using neuron-specific targeted deletion of D2Rs. Deletion of Chl-D2Rs (Chl-Drd2KO) impaired LTD induction in both subtypes of MSNs. LTD induction was restored in the Chl-Drd2KO mice by an M1-selective muscarinic acetylcholine receptor antagonist. In contrast, after the deletion of iMSN-D2Rs (iMSN-Drd2KO), LTD induction was intact in MSNs. Separate interrogation of direct pathway and iMSNs revealed a deficit in LTD induction only at synapses onto iMSNs that lack D2Rs. LTD induction in iMSNs was restored by D2R agonist application. Our findings suggest that Chl D2Rs strongly modulate LTD induction in MSNs, with iMSN-D2Rs having a weaker, iMSN-specific, modulatory effect.

Graphical Abstract
In Brief

The cellular location of dopamine D2 receptors (D2Rs) involved in corticostriatal long-term synaptic depression (LTD) is controversial. Augustin et al. show that D2Rs on cholinergic interneurons strongly modulate LTD induction at synapses onto all medium spiny neurons (MSNs), while D2Rs on iMSNs weakly modulate nduction at synapses onto iMSNs.

INTRODUCTION

The dorsal striatum (DS) is the main input structure of the basal ganglia, which controls action selection and action learning. Dopaminergic signaling has crucial roles in proper DS function (Albin et al., 1989; DeLong, 1990), as highlighted by the severe movement problems caused by dopamine (DA) neuron loss in Parkinson’s disease (PD). The DS receives convergent glutama-tergic inputs from the cortex and thalamus that form synapses on medium spiny neurons (MSNs), striatopallidal indirect pathway MSNs (iMSNs), and striatonigral direct pathway MSNs (dMSNs), which differ in their axonal projection targets and DA receptor expression. At corticostriatal synapses, DA regulates synaptic transmission (Calabresi et al., 1997a; Kreitzer and Malenka, 2005; Pawlak and Kerr, 2008; Reynolds and Wickens, 2002; Wang et al., 2006). Loss of DA signaling at these synapses results in aberrant synaptic transmission that likely contributes to functional changes in the basal ganglia circuitry underlying motor dysfunctions in many neurodegenerative disorders, including PD (Beeler et al., 2012; Calabresi et al., 2007; Kreitzer and Malenka, 2007; Shen et al., 2008).

In the dorsolateral striatum (DLS), the best characterized and most reproducible form of synaptic plasticity is endocannabinoid-mediated long-term depression (eCB-LTD). Striatal eCB-LTD requires the combined activation of \(G_{i/o}\)-coupled DA D2 receptors (D2Rs) and \(G_q\)-coupled metabotropic glutamate type 5 (mGluR5) receptors and L-type Ca\(^{2+}\) channels. Co-activation of these receptors and channels leads to the production and release of...
postsynaptic eCBs that bind to presynaptic CB1 receptors to induce LTD (Calabresi et al., 1994, 1997b; Choi and Lovinger, 1997; Gerdeman et al., 2002; Sung et al., 2001). Although D2R involvement in LTD induction is clear, the cellular locus of these D2Rs has been debated (Calabresi et al., 1992; Kreitzer and Malenka, 2005; Tozzi et al., 2011; Wang et al., 2006). These receptors are localized on different striatal cells, iMSNs, and local cholinergic interneurons (Chls), as well as on glutamatergic and DAergic afferents (Bamford et al., 2004; Gerfen et al., 1990; Maurice et al., 2004).

The D2Rs expressed on Chls have been implicated in LTD induction based on pharmacological studies. Activation of Chl D2Rs decreases acetylcholine (ACh) release, which then decreases the activation of the M1 receptors localized on MSNs, disinhibiting L-type Ca\(^{2+}\) channels (Bernard et al., 1992; Hersch et al., 1994; Maurice et al., 2004; Wang et al., 2006; Yan et al., 1997). The resulting increases in calcium influx through L-type Ca\(^{2+}\) channels enhance the mobilization of eCBs to induce LTD (Calabresi et al., 1994; Choi and Lovinger, 1997; Gerdeman et al., 2002; Wang et al., 2006). This ACh interaction with M1 receptors facilitates LTD induction at both MSN subtypes (Wang et al., 2006).

There is also evidence that D2Rs on iMSNs contribute to LTD, including the finding that LTD induction requires the activation of D2Rs plus the lack of adenosine A\(_{2A}\) (A2A) receptor activation (Shen et al., 2008; Tozzi et al., 2011). These two receptors are expressed on iMSNs and are oppositely coupled to cyclic AMP (cAMP) production, which affects LTD induction (Shen et al., 2008). Low levels of intracellular cAMP are permissive for LTD induction, whereas high levels inhibit LTD induction (Augustin et al., 2014). The activation of A2A receptors leads to increased cAMP signaling and protein kinase A (PKA) activation, which then inhibits mGluR5 signaling via the regulator of G protein signaling type 4 (RGS4), preventing the mobilization of eCB and ultimately inhibiting LTD. When cAMP levels are low, RGS4 does not inhibit mGluR5 receptor activity, allowing eCB mobilization (Lerner and Kreitzer, 2012). Given that this signaling is restricted to iMSNs, the involvement of iMSN D2Rs in LTD will be restricted to this MSN subtype. A few reports have suggested that this is the case (Kreitzer and Malenka, 2005; Shen et al., 2008; Trusel et al., 2015). However, eCB-LTD at synapses onto dMSNs has been observed (Bagetta et al., 2011; Picconi et al., 2011; Shen et al., 2008; Wang et al., 2006, 2017; Wu et al., 2015) and is prevented by the D2R antagonist in these neurons (Bagetta et al., 2011; Wang et al., 2006). It seems unlikely that iMSN D2Rs can account for all of the roles of the receptor in LTD induction. The roles in eCB-LTD of different cellular D2R pools cannot be resolved using standard receptor ligands because these agents affect all of the receptors in a slice preparation. Thus, to determine which D2Rs are implicated in LTD induction, we used gene targeted deletion to selectively remove D2Rs from iMSNs and Chls. Our findings provide evidence that Chl D2Rs contribute to LTD induction in both dMSNs and iMSNs, while D2Rs on iMSNs can further regulate LTD induction at synapses onto that MSN subtype. These findings help to clarify the role of different D2R subpopulations in the modulation of LTD, thus unifying previous findings.
RESULTS

Selective Deletion of D2Rs in iMSNs and Chls

We bred mice containing loxP sites flanking exon 2 of the Drd2 gene with Adora2A Cre+/− or ChAT Cre+/− mice to selectively remove the D2Rs only after Cre-mediated recombination in iMSNs (iMSN-Drd2 knockout [KO] mice) and Chls (Chl-Drd2KO mice), respectively (Figures 1A–1C). To confirm that unexpected recombination had not occurred in striatal cell types other than those targeted, qPCR was performed to examine mRNA levels. As expected, there was no significant reduction in striatal D2R mRNA in the Chl-Drd2KO mice (Figure 1D; p > 0.05). Chls make up a small fraction of cells in the striatum (Goldberg and Wilson, 2017), making it impossible to resolve the reduction of D2R mRNA levels in the Chl-Drd2KO mice using qPCR. These results show that germline recombination has not occurred in these mice because the majority of the striatal D2R mRNA is still present. In the iMSN-Drd2KO mice, there was a 90% reduction in striatal D2R mRNA compared to Drd2loxP/loxP, which is similar to a previous report (Figure 1E; p < 0.0001; Lemos et al., 2016).

To functionally assess Chl D2R deletion, we examined the DA-mediated “pause” in the firing of Chls in wild-type (WT) and Chl-Drd2KO mice. In cell-attached recordings, we measured the baseline firing rate of Chls in the DS, which was not different between the two genotypes (ChAT Cre: 2.53 ± 0.25, Chl-Drd2KO: 2.06 ± 0.25, p = 0.24, Student’s t test). Following bath application of (N)-1-(2-nitrophenyl)ethyl (NPEC)-caged DA (100 µM), a 1-s UV light pulse caused a pause in the tonic firing of Chls in ChAT Cre mice but not in Chl-Drd2KO mice (Figure 2A). This pause was blocked by 5 µM sulpiride, a D2R antagonist (Figures 2A and 2B). UV light (385 nm) did not affect the tonic firing of Chls in the absence of caged DA, assessed during the baseline recording period (Figure 2A). In addition, sulpiride increased the firing rate of Chls in ChAT Cre cells (2.53 ± 0.25–3.52 ± 0.46 Hz; p = 0.02, paired t test) but not Chl-Drd2KO cells (Figure 2C; 2.06 ± 0.25–1.96 ± 0.27 Hz; p = 0.69). These molecular and physiological results indicate the absence of functional D2Rs from our iMSN- and Chl-Drd2KO mice.

Intact D2 Autoreceptor Function in iMSN and Chl Drd2KO Mice

To investigate whether D2R deletion had an off-target effect on D2 autoreceptors on DA terminals, we performed fast-scan cyclic voltammetry (FSCV) in the DLS. There was no difference in quinpirole-induced inhibition of DA release at two concentrations in iMSN-Drd2KO mice compared to Drd2loxP/loxP (Figures 3A–3C; repeated-measures ANOVA: drug concentration, F1,6 = 136.7, p ≤ 0.0001; drug concentration × genotype interaction, F1,6 = 1.35, p = 0.29), consistent with a previous report (Lemos et al., 2016). To assess D2 autoreceptor modulation in the Chl-Drd2KO mice in the absence of nicotinic ACh receptor (nAChR) regulation and eliminate any role of D2 effects on ACh release (Rice et al., 2011; Threlfell et al., 2010), dihydro-β-erythroidine hydrobromide (DHβE) (500 nM), a β2*-nAChR antagonist, was co-applied with quinpirole. The quinpirole-induced inhibition of DA release in the presence of DHβE was similar in both genotypes (Figures 3D–3F; repeated-measures ANOVA [rmANOVA]: drug concentration, F1,9 = 71.47, p ≤ 0.0001; drug
concentration × genotype interaction, $F_{1,9} = 1.59, p = 0.24$). Thus, D2 autoreceptor function was intact in both mouse models, supporting the specificity of our receptor deletion strategy.

**Loss of LTD in Chl-Drd2KO Mice in Field Potential Recordings**

To directly test whether D2 modulation of LTD induction is mediated by receptors on Chls, we first measured the synaptically driven population spike (PS) in the DLS using non-invasive extra-cellular recordings designed to preserve intracellular signaling from slices obtained from Chl-Drd2KO mice and Drd2$^{loxP/loxP}$ controls. We used intrastriatal high-frequency stimulation (HFS) to induce eCB-LTD. This protocol induced a robust long-lasting depression of PS amplitude in Drd2$^{loxP/loxP}$ (Figure 4A; 81% ± 3%, $p < 0.05$), but not in the Chl-Drd2KO mice (Figure 4A; 101% ± 3%, $p > 0.05$; rmANOVA, time × genotype interaction, $F_{49,637} = 1.76, p = 0.0015$). The LTD magnitude is similar to that in published data from our laboratory in adult mice (Atwood et al., 2014). In controls, LTD induction was blocked by a CB$_1$ receptor antagonist, AM251 (3 µM; Figure 4B; 101% ± 3%, $p > 0.05$), consistent with previous reports that LTD is dependent on CB$_1$ receptor activation (Choi and Lovinger, 1997). LTD was also blocked by the D2 receptor antagonist sulpiride (5 µM; Figure 4C; 95% ± 5%, $p > 0.05$). These results suggest that Chl D2Rs are required for the induction of eCB-LTD.

The HFS-LTD in mice with iMSN D2R deletion did not differ in magnitude or kinetics from that observed in controls (Figure 4D; iMSN-Drd2KO: 78% ± 6%, $p < 0.05$; Drd2$^{loxP/loxP}$: 82% ± 4%, $p < 0.01$; rmANOVA: time × genotype interaction, $F_{1,17,677} = 0.97, p = 0.57$). Bath application of AM251 (3 µM) blocked LTD induction in both iMSN-Drd2KO (109% ± 10%, $n = 5, p > 0.05$) and Drd2$^{loxP/loxP}$ (110% ± 8%, $p > 0.05$) mice (Figure 4E). Sulpiride blocked LTD induction in both genotypes, indicating D2R involvement (Figure 4F; iMSN-Drd2KO: 100% ± 7%, $p > 0.05$; Drd2$^{loxP/loxP}$: 105% ± 5%, $p > 0.05$).

**LTD Induction at Synapses onto MSNs Is Dependent on Chl D2Rs**

To further determine whether Chl D2Rs are required for eCB-LTD induction at glutamatergic MSN synapses, we conducted whole-cell recordings. HFS was paired with postsynaptic depolarization (0 mV), and excitatory postsynaptic current (EPSC) amplitude was measured. Similar to the results obtained from field potential recordings, HFS-induced LTD was absent in slices from Chl-Drd2KO mice (Figure 5A; 100% ± 2%, $p > 0.05$), but was intact in controls (Figure 5A; 80% ± 4%, $p < 0.05$). The magnitude and type of synaptic plasticity that occurred in response to HFS varied in the controls, which is in agreement with previous reports (Figure 5B) (Kreitzer and Malenka, 2007; Sung et al., 2001). This heterogeneity in the individual cellular response was lost in the Chl-Drd2KO mice (Figure 5B). There was a significant difference between genotypes in the EPSC amplitude after HFS (rmANOVA: time × genotype interaction, $F_{49,490} = 3.71, p < 0.0001$). This loss of LTD was observed in both subpopulations of MSNs in the Chl-Drd2KO mice (Figures 5C and 5D; dMSNs: 106% ± 9%, $p > 0.05$; iMSNs: 105% ± 6%, $p > 0.05$), whereas LTD induction was intact in litter-mate controls (Figures 5C and 5D; dMSNs: 77% ± 7%, $p < 0.05$; iMSNs: 79% ± 7%, $p < 0.05$). LTD induction was blocked by AM251 (3 mM) (Figure 5E; 93% ± 6%, $p > 0.05$), indicating CB$_1$ dependence. Bath application of sulpiride (5 mM) also blocked LTD in control slices (Figure 5F; 102% ± 6%, $p > 0.05$). D2R activation paired with the HFS...
protocol enhances LTD (Augustin et al., 2014). If LTD induction is mediated by D2Rs located on iMSNs, then the addition of quinpirole (5 µM), a D2R agonist, should restore HFS LTD in the Chl-Drd2KO mice. In the presence of quinpirole, HFS-induced LTD was observed in the Drd2loxP/loxP mice (Figure 5G; 71% ± 10%, p < 0.05), but not in the Chl-Drd2KO mice (Figure 5G; 95% ± 9%, p > 0.05). This suggests that dopaminergic modulation of LTD in MSNs is dependent on Chl D2Rs and not iMSN D2Rs.

**LTD Induction Downstream of D2Rs Is Intact in Chl-Drd2KO Mice**

To determine whether the absence of LTD in the Chl-Drd2KO mice was due to a deficit in eCB mobilization downstream of the D2Rs, a D2R independent but eCB dependent form of LTD was induced using a group 1 metabotropic glutamate receptor (mGluR) agonist, (RS)-3,5-Dihydroxyphenylglycine (DHPG) (100 µM) (Kreitzer and Malenka, 2005; Wu et al., 2015). When cells were voltage clamped at 50 mV, bath application of DHPG for 10 min elicited LTD in both Drd2loxP/loxP (Figure 5H, 78% ± 2%, p < 0.01) and Chl-Drd2KO mice (Figure 5H, 83% ± 5%, p < 0.05). The LTD was similar in magnitude and kinetics in the Chl-Drd2KO and controls (rmANOVA: time 3 genotype interaction, F48,528 = 0.68, p = 0.95). This DHPG-induced LTD was blocked by AM251 in both genotypes (Figure 5I; Drd2loxP/loxP, 92% ± 8%, p > 0.05; Chl-Drd2KO, 94% ± 6%, p > 0.05). Thus, eCB-LTD can be induced at synapses onto MSNs in Chl-Drd2KO mice by engaging signaling downstream of the D2Rs.

**M1 Antagonist Restores LTD in Chl-Drd2KO Mice**

Previous reports have implicated Chl D2Rs in LTD induction, presumably due to decreased ACh release and reduced M1 receptor inhibitory actions on L-type calcium channels, thereby promoting LTD (Wang et al., 2006). However, there is still considerable controversy surrounding this proposed mechanism. We tested whether M1 antagonism would restore LTD induction in the Chl-Drd2KO striatum. In the presence of VU 0255035 (1 µM), HFS-induced LTD was restored in the Chl-Drd2KO mice (Figure 5J; 70% ± 8%, p < 0.05). This LTD was blocked in the presence of AM251 (3 µM, Figure 5K; 104% ± 18%, p > 0.05), confirming the role of eCB signaling. These results further provide direct evidence that D2R-mediated M1 receptor inhibition facilitates eCB-LTD.

**HFS Induces LTD at MSN Synapses in iMSN-Drd2KO Mice**

To determine whether iMSN D2Rs are also required for LTD at synapses onto MSNs, we performed whole-cell voltage clamp recordings in the iMSN-Drd2KO mice. HFS induced LTD in Drd2loxP/loxP (Figure 6A; 81% ± 4%, p < 0.01). Although HFS did not significantly induce LTD in the iMSN-Drd2KO mice (Figure 6A; 85% ± 9%, p > 0.05), 6/7 cells showed a significant depression after HFS (Figure 5B; Mann-Whitney, each <0.05). There was heterogeneity in the type and magnitude of synaptic plasticity displayed in the individual cells in the Drd2loxP/loxP mice and to a lesser extent in the iMSN-Drd2KO mice. There was no difference in the EPSC amplitude after HFS between genotypes (Figure 6B; Mann-Whitney, p > 0.05). Because some cells in the iMSN-Drd2KO mice showed a reduction in EPSC amplitude after HFS, we further tested whether cells in these animals were sensitive to the same pharmacological agents as the controls. The HFS-LTD was prevented by AM251 (Figure 6C; Drd2loxP/loxP; 95% ± 9%, p > 0.05; iMSN-Drd2KO: 92% ± 6%, p > 0.05) and
sulpiride (Figure 6D; Drd2loxP/loxP: 97% ± 9%, p > 0.05; iMSN-Drd2KO: 95% ± 10%, p > 0.05) in both genotypes. Bath application of quinpirole (5 mM) paired with HFS resulted in LTD in both genotypes (Figure 6E; Drd2loxP/loxP: 68% ± 7%, p < 0.05; iMSN-Drd2KO: 61% ± 3%, p < 0.05). There was an increase in the magnitude of LTD in the iMSN-Drd2KO mice in the presence of quinpirole compared to conditions without the drug (Figures 6A and 6E; Mann-Whitney, p < 0.01). Thus, removal of iMSN D2Rs impairs LTD induction at MSN synapses, although not in all cells when recording from a mixed population of MSNs, both iMSNs and dMSNs.

**mGluR5 Activation Induces LTD in iMSN-Drd2KO Mice**

We next determined whether DHPG-induced LTD was altered in the iMSN-Drd2KO mice. Bath application of DHPG (100 mM) elicited LTD, similar in magnitude and kinetics, in both genotypes (Figure 6F; Drd2loxP/loxP: 75% ± 3%, p < 0.05; iMSN-Drd2KO: 69% ± 5%, p < 0.05). DHPG-induced LTD was prevented in the presence of a CB1 receptor antagonist, AM251 (3 µM), in both the Drd2loxP/loxP (Figure 6G; 91% ± 4%, p < 0.05) and iMSN-Drd2KO (Figure 6G; 100% ± 9%, p < 0.05) mice. Our data show that DHPG-LTD induction is intact after the conditional deletion of iMSN D2Rs.

**LTD Is Lost at Inputs onto iMSNs but Not dMSNs in the iMSN-Drd2KO Mice**

Previous findings showed that HFS produces long-term potentiation (LTP) at MSN synapses in mice that lack D2Rs on all cell types (Calabresi et al., 1997b). It is possible that the deletion of D2Rs on iMSNs may result in LTP at synapses onto iMSNs. To directly test this hypothesis, we recorded from dMSNs in a Drd1-TdTomato reporter mouse and non-fluorescently labeled MSNs, presumably iMSNs. In controls, HFS induced LTD at synapses onto dMSNs (Figure 7A; 81% ± 1%, P < 0.01) and iMSNs (Figure 7B; 77% ± 8%, p < 0.05), consistent with previous findings that LTD can be induced in both types of MSNs (Picconi et al., 2011). In striatal slices from iMSN-Drd2KO mice, HFS elicited LTD in dMSNs (Figure 7A; 83% ± 5%, p < 0.05), but not in the iMSNs (Figure 7B; 100% ± 6%, p > 0.05). Synapses on iMSNs exhibited LTD with a prevalence of 40% (4/10), while those on dMSNs showed an LTD prevalence of ~70% (8/11) in iMSN-Drd2KO mouse slices. These results indicate that D2Rs on iMSNs weakly modulate LTD induction at synapses onto this MSN subtype, but loss of iMSN D2Rs does not prevent LTD altogether. In the presence of quinpirole (5 mM), HFS induced LTD in both dMSNs and iMSNs in the Drd2loxP/loxP (dMSN, Figure 7C; 76% ± 4%, p < 0.05; iMSN, Figure 7D; 75% ± 5%, p < 0.05) and iMSN-Drd2KO mice (dMSN, Figure 7C; 80% ± 3%, p < 0.01; Figure 7d; 64% ± 6%, p < 0.001). The prevalence of LTD increased from 40% to ~90% in iMSN-Drd2KO mice. Thus, iMSN D2Rs contribute to LTD only in iMSNs, but in the absence of these receptors Chl D2Rs appear to facilitate LTD induction in iMSNs.

**DISCUSSION**

We have dissected the roles of different cellular D2R populations in corticostriatal LTD. Chl D2Rs are required for LTD induction, and M1 antagonism can restore LTD in the absence of these receptors. The restoration of LTD with a selective M1 antagonist provides even stronger support for the role of this receptor in comparison to previous studies that used a
less selective antagonist (Bagetta et al., 2011; Wang et al., 2006). Our findings are in agreement with previous reports indicating that Chl D2Rs facilitate LTD induction, presumably through reduced ACh release and subsequent M1 receptor actions (Wang et al., 2006). These findings support the idea that D2Rs modulate the core mechanisms involved in LTD induction, most likely by boosting eCB production (Giuffrida et al., 1999; Kreitzer and Malenka, 2005). Removal of iMSN D2Rs did not abolish LTD induced in the over-all MSN population, but only at synapses onto iMSN synapses. D2R agonist activation, presumably by activating Chl D2Rs, restored LTD in iMSNs in the iMSN-Drd2KO mice. Our data suggest that D2R-cAMP signaling has a modulatory role in the induction of iMSN LTD, which is consistent with previous findings (Augustin et al., 2014; Kreitzer and Malenka, 2005). We have confirmed and extended previous findings on the role of D2Rs in striatal LTD by identifying two distinct cellular pathways for the modulation of LTD induction, both of which require the activation of D2 and CB1 receptors. The lack of HFS-induced synaptic modification was not attributed to off-target effects such as differences in DA autoreceptor function. More specifically, our findings show that Chl D2Rs strongly modulate LTD in MSNs via M1 receptors, with the iMSN D2Rs having a weaker, cell-type specific modulatory effect on LTD induction.

It has been speculated that eCB LTD can be induced more reliably at iMSN synapses than dMSN synapses (Kreitzer and Malenka, 2007), and this may depend on induction conditions (Shen et al., 2008; Trusel et al., 2015). This topic has remained controversial despite several reports of LTD occurring in both MSN subtypes (Bagetta et al., 2011; Picconi et al., 2011; Shen et al., 2008; Wang et al., 2006, 2017; Wu et al., 2015). We showed that LTD can be induced in both dMSNs and iMSNs using HFS, which is consistent with previous findings (Wang et al., 2006, 2017).

Although the rate and timing of neuronal activity may be important for information processing, there is no consensus on a reliable D2R-dependent LTD-producing spike-timing dependent plasticity (STDP) protocol among the few groups that have used this protocol to assess corticostriatal plasticity (Fino et al., 2005; Nazzaro et al., 2012; Pawlak and Kerr, 2008; Shen et al., 2008, 2016). As a result, we decided to use a conventional stimulation protocol, HFS, which has been used by many laboratories and reliably induces D2R-dependent LTD (Augustin et al., 2014; Calabresi et al., 1994; Choi and Lovinger, 1997; Kreitzer and Malenka, 2005; Lerner and Kreitzer, 2012; Wang et al., 2006). No LTD induction protocol in brain slices can replicate precisely what occurs in vivo, and thus the purpose of brain slice experiments is to determine whether and when particular mechanisms contribute to plasticity. We noted that the magnitude of LTD in the present study is smaller than in our previous reports (Choi and Lovinger, 1997). We believe that this is mainly due to the use of adult mice in the present study, while young animals were examined in previous studies (Choi and Lovinger, 1997; Gerdeman et al., 2002; Sung et al., 2001). We have previously reported that LTD becomes weaker as animals transition from pre-adolescence to maturity (Partridge et al., 2000). In our most recent study examining adult mice, we observed LTD with a magnitude comparable to that observed in the present study (Atwood et al., 2014). Regardless of the magnitude of depression, it is clear that the LTD reported herein involves the same molecular mechanisms as reported previously.
The role of D2R signaling in modulating corticostriatal plasticity and dopamine dependent behaviors is well established (Baik et al., 1995; Calabresi et al., 1997a; Kreitzer and Malenka, 2007; Shen et al., 2008). A recently published study showed that disruption in iMSN D2R signaling is associated with motor impairments (Lemos et al., 2016). The impaired modulation of LTD in iMSNs in these mice could contribute to these motor deficits. The dysregulation of excitatory synaptic transmission, combined with an enhancement in inhibitory synaptic transmission, as described previously (Lemos et al., 2016), may contribute to the motor deficits observed in iMSN-Drd2KO mice. The balance between ACh and DA signaling is important for basal ganglia function (Aosaki et al., 2010), and our findings indicate one mechanism affected by this balance.

eCB-LTD is important for shaping neural circuits to regulate various behaviors (Augustin and Lovinger, 2018). LTD induction at glutamatergic synapses onto MSNs is Chl D2R selective and pathway independent, favoring induction in both dMSNs and iMSNs in response to relatively strong combined glutamatergic and DAergic afferent activation. Induction of LTD may help to oppose LTP at glutamatergic synapses onto MSNs or reset the overall striatal network during action selection and motor planning. Another function of LTD may be to weaken individual inputs promoting the need for greater convergence among cortical in-puts to reach threshold in a given MSN. This may help to shape action production or selection by favoring output only in response to a threshold level of cortical input. The CB₁ receptors at orbitofrontal cortex (OFC) synapses onto MSNs appear to play a key role in habit learning (Gremel et al., 2016). Thus, LTD at these synapses may have key roles in suppressing goal-directed actions that involve OFC input, favoring behavioral control by other corticostriatal afferents. Given that activation of dMSNs is often associated with action production, it is quite possible that CB₁ receptors on cortical inputs to dMSNs may be part of the suppression of signals that drive goal-directed actions. Tonic DA stimulation of D2Rs may facilitate motor as well as cognitive functions (Berke and Hyman, 2000; Schultz, 2007). We further extend this hypothesis by proposing that iMSN D2R-selective LTD induction by tonic DA signaling and weak afferent activation may signal real-time changes in the contingency and reinforcement value during action selection. Selectively suppressing the cortical drive on the indirect pathway under this circumstance may allow for the performance of action sequences through the unaltered cortical input to dMSNs. Aberrant changes in DA signaling iMSN-specific LTD induction and cortical synaptic strength may underlie many movement disorders, such as PD.

**EXPERIMENTAL PROCEDURES**

**Animals**

The animal care and experimental procedures used were approved by the Institutional Animal Care and Use Committee of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and performed in accordance with the NIH care and use of animals guidelines. Mice were group housed and maintained on a 12/12-hr light-dark cycle. Male and female mice were used for whole-cell voltage clamp (4–8 weeks old) and field potential (8–16 weeks old) recordings.

*Cell Rep. Author manuscript; available in PMC 2018 October 12.*
Deletion of D2Rs from iMSNs and Chls

Mice carrying a “floxed” exon 2 dopamine D2 (Drd2) gene (Bello et al., 2011) were bred with hemizygous mice expressing the Cre recombinase under the control of iMSN-active A2A (Adora2a, Mutant Mouse Resource & Research Centers [MMRRC]) or ChAT (ChAT-IRES-Cre knockin; Jackson Laboratory) promoter to selectively remove D2Rs from iMSNs or Chls, respectively. A subset of iMSN- and Chl-Drd2KO mice were bred with Drd1a-tdTomato mice (Jackson Laboratory) (Ade et al., 2011).

qPCR

Striatal RNA was isolated and purified using the RNeasy Lipid Tissue Mini Kit (QIAGEN), according to the manufacturer’s instructions. RNA quality and quantity were evaluated using a NanoDrop UV-Vis Spectrophotometer (Thermo Scientific). RNA was reverse transcribed into cDNA using the QuantiTect Reverse Transcription Kit (QIAGEN), according to the manufacturer’s instructions. qPCR was performed using a StepOnePlus system (Thermo Scientific), with each sample repeated in triplicate. Each 20-µL reaction contained 10 µL TaqMan Fast Advanced Master Mix 2x, 1 µL pre-designed gene assay probes (Drd2, Mm00438541_m1; actin beta (Actb), Mm00607939_S1), 4 µL 2.5 ng/µL cDNA, and 5 µL RNase/DNase-free water (all from Life Technologies). The qPCR program consisted of 50º C for 5 min, followed by 10 min at 95º C, and then 40 cycles of 15 s at 95º C and 1 min at 60º C. PCR amplification plots were analyzed using the comparative C_T method (Schmittgen and Livak, 2008). Triplicate C_T values were averaged for each gene before calculating the ΔC_T.

Slice Preparation and Solutions for Electrophysiology and FSCV

For whole-cell voltage clamp recordings and FSCV, mice were anesthetized with isoflurane and rapidly decapitated. Brains were removed and immediately submerged into ice-cold oxygenated (95% O_2 and CO_2) sucrose-substituted artificial cerebrospinal fluid (ACSF) containing the following (in mM): 30 NaCl, 4.5 KCl, 1 MgCl_2, 10 glucose, 1.2 NaH_2PO_4, 26 NaHCO_3, and 194 sucrose. For cell-attached recordings in Chls, mice were intracardially perfused using sucrose-ACSF after anesthesia. Coronal slices (250–300 µm) containing the striatum were prepared using a vibratome (Leica). Brain slices for whole-cell physiology experiments were transferred and incubated for 30–60 min at 32 C in oxygenated recording ACSF containing the following (in mM): 124 NaCl, 4.5 KCl, 1 MgCl_2, 2 CaCl_2, 10 D-glucose, 1.2 NaH_2PO_4, and 26 NaHCO_3. For cell-attached recordings, slices were incubated for 30 min in ACSF containing the following (in mM): 126 NaCl, 2.5 KCl, 1.2 MgCl_2, 25 NaHCO_3, 1.4 NaH_2PO_4, 10 glucose, 2.4 CaCl_2, and 0.35 ascorbic acid. For FSCV, brain slices were incubated for 30–60 min at 32º C in oxygenated voltammetry recording ACSF (pH 7.4) containing the following (in mM): 126 NaCl, 2.5 KCl, 1.2 MgCl_2, 2.4 CaCl_2, 1.2 NaH_2PO_4, 25 NaHCO_3, 10 dextrose, 20 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), and 0.4 L-ascorbic acid. After at least 30 min of incubation at 32 C, slices were moved to room temperature until used for recordings. All of the physiology and voltammetry experiments were conducted in a submersion chamber perfused (1.5–2 mL/min) with the appropriate recording solution (30º–32º C).
Cell-Attached Recordings

Slices were visualized using an upright microscope (Scientifica, Uckfield, East Sussex, UK) and a 40x (0.8 numerical aperture) water immersion objective (Olympus, Waltham, MA). Patch pipettes (2–4 MΩ resistance) were pulled on a P-97 Sutter puller (Sutter Instrument, Novato, CA) from borosilicate glass capillaries (1.5 mm outer diameter, 0.86 mm inner diameter, World Precision Instruments, Sarasota, FL) and filled with internal solution containing the following (in mM): 140 K-gluconate, 0.1 CaCl₂, 2 MgCl₂, 10 HEPES, 2 Mg-ATP, 0.1 Na-guanosine triphosphate (GTP), 10 phosphocreatine, and 10 1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) for uncaging experiments (Figure 2). ACSF was used in place of internal solution in some cell-attached recordings. For optical uncaging experiments, 385 nm UV light (1 s) was delivered via whole-field illumination using a high-power (>95 mW) light-emitting diode (LED) source (LED4D067, Thorlabs). A total of 100 µM NPEC-caged dopamine (Tocris Bioscience, Bristol, UK) was bath applied. Data were collected using a Multiclamp 700B amplifier, a Digidata 1322A, and Clampex version 9.2 software (Molecular Devices, Sunnyvale, CA).

FSCV

Evoked DA release was measured using FSCV in brain slices with the DEMON voltammetry and analysis software (Yorgason et al., 2011). Glass-encased carbon fiber (80–150 M exposed length) electrodes were made by aspirating a single carbon fiber into a glass capillary (A-M Systems). The carbon fiber electrode was placed in the DLS near the border of the external capsule to measure DA release. The carbon fiber was held at 0.4 V and increased to 1.2 V and back at a scan rate of 400 V/s every 100 ms (10 Hz), with a triangle wave generated using Chem-Clamp (Dagan Corporation). A twisted bipolar electrode (Plastics One) was used to generate electrical pulse stimulation (400–500 µA with a 2-ms pulse duration) every 5 min. The ADS3 constant current stimulator (Digitimer) was used for all of the electrical stimulation experiments, FSCV, and physiology. Before recording, carbon fiber electrodes were conditioned at 60 Hz for 5–10 min. When five consecutive stable DA transients were recorded, drugs were bath applied and the responses recorded.

Field Potential Recordings

Extracellular recordings were conducted in the presence of the GABA_A receptor antagonist picrotoxin (50 µM), with micropipettes (2.0–3.5 MΩ) filled with 1 M NaCl. A twisted bipolar electrode (Plastics One) was placed in DLS near the border of the external capsule. The stimulus intensity was adjusted to yield an evoked PS amplitude that was half the amplitude of the maximal evoked response. The half-maximal response (0.5–1.0 mV) was evoked with a stimulus intensity of 0.3–0.9 mA. A test stimulus was given every 30 s with a duration of 40 µs. After a stable baseline was achieved, HFS consisting of four 1 s 100 Hz trains delivered every 10 s was given.

Whole-Cell Voltage Clamp Recording

Cells were visualized using differential interference contrast microscopy (40x/0.80 numerical aperture [NA] water immersion objective) with an Olympus upright microscope (BX51W1) and a digital charge-coupled device (CCD) camera (DAGE-MTI). The cells were...
displayed on a video monitor (Sony) in real time to aid in the placement of the stimulating and recording electrodes. To visualize dMSNs in the Drd1a-tdTomato-iMSN-Drd2KO mice, epifluorescence microscopy with excitation via an X-Cite 120 LED (Excelitas Technologies, Waltham, MA) was used with a CY3 filter. Otherwise, MSNs were identified based on their membrane resistance, capacitance, and size. Recording pipettes (3–5 MΩ) were filled with a cesium methanesulfonate-based internal solution containing the following (in mM): 120 CsMeSO₃, 10 tetraethylammonium (TEA)-Cl, 10 HEPES, 5 NaCl, 5 QX-314, 4 Mg-ATP, 1.1 EGTA, and 0.3 Na-GTP. Whole-cell voltage clamp recordings were performed at −70 mV (holding potential), unless otherwise indicated. All of the experiments were conducted in the presence of 50 µm picrotoxin to isolate glutamatergic transmission. For recordings with electrical stimulation, a tungsten bipolar stimulating electrode (FHC) with a tip separation of 505 µm was placed in the DLS near the external capsule adjacent to the recording site. Cells received paired stimuli separated by 25 or 50 ms every 20 s with a duration of 40 µs. After 10 min of stable EPSC recording, HFS (four 1 s 100 Hz trains delivered every 10 s) was delivered, while the MSN was depolarized to 0 mV for LTD induction. The series resistance (<20 MΩ) was monitored throughout the recording with a 10-mV, 100-ms depolarizing step after the last evoked EPSC, and cells that had changes >15% were excluded from analysis. Recordings were collected using a Multiclamp 700B amplifier (Molecular Devices). The membrane currents were filtered at 2 kHz and digitized at 10 kHz using Digidata 1332A (Molecular Devices). Data were acquired using pCLAMP version 10.3 and analyzed using Clampfit version 10.3 (Molecular Devices).

Statistical Analysis
Statistical analyses were performed using GraphPad Prism version 7 software. Data were analyzed using paired and unpaired Student’s t tests, rmANOVA, the nonparametric Mann-Whitney rank-sum test, and the Wilcoxon signed rank test. Summary data are reported as the means ± SEMs, and p < 0.05 was considered statistically significant.

Drugs
Drugs were prepared in a stock solution of water or DMSO and diluted to their final concentration in ACSF. The final concentration of DMSO was <0.1%. β-Cyclodextrin (final concentration <0.001%; TCI America) was used as a carrier for AM251 delivery. (−)-Quinpirole hydrochloride, (RS)-3,5-DHPG, DHβE, VU 0255035, NPEC-caged dopamine, and AM251 were purchased from Tocris Bioscience. (±)-Sulpiride and picrotoxin were purchased from Sigma.

ACKNOWLEDGMENTS
We thank Lucas Voyvodic for assistance with the electrophysiological experiments during the paper revision. This work was supported by the NIAAA Division of Intramural Clinical and Biological Research (ZIA AA000407).

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Highlights

- Long-term depression (LTD) occurs at glutamatergic synapses on all striatal MSNs
- D2 receptors on cholinergic interneurons strongly modulate LTD in all MSNs
- D2 receptors on iMSNs can further modulate LTD at iMSN-specific synapses
- M1 muscarinic ACh receptors mediate ACh suppression of LTD
Figure 1. Selective Deletion of D2Rs in iMSNs and Chls

(A) Schematic representation of Cre-mediated recombination depicting the removal of exon 2, which is flanked by loxP sites, of the Drd2 gene.

(B and C) Diagram of breeding strategy used to generate (B) Chl-Drd2KO and (C) iMSN-Drd2KO mice.

(D) Data reported as mean ± SEM. There was no significant change in D2R mRNA expression levels after D2R deletion from Chls (n = 8–10 mice).

(E) iMSN D2R deletion resulted in an 90% reduction in D2R mRNA levels in the iMSN-Drd2KO mice (n = 3–4 mice).
Figure 2. D2R-Mediated Pausing in Chls Is Lost in Chl-Drd2KO Mice

(A) Example cell-attached recordings from Chls from wild-type (ChAT Cre, left) or knockout (Chl-Drd2KO, right) mice. Top: 1 s of UV light (385 nm, violet line) had no effect on the tonic firing rate of Chls in ACSF. Middle: In the presence of 100 µM NPEC-caged dopamine, UV light caused a pause in the tonic firing of Chls in wildtype but not knockout animals. Bottom: This pause was blocked by 5 µM sulpiride.

(B) Data reported as mean ± SEM. The dopamine-mediated pause, or latency to fire, following the light pulse in ChAT Cre mice was 0.97 ± 0.11 s, which was reduced to 0.49 ± 0.08 s in sulpiride (p < 0.001, paired t test). This pause was absent in Chl-Drd2KO animals (latency to fire baseline: 0.74 ± 0.08 s; in sulpiride: 0.7 ± 0.11; p = 0.75, paired t test).

(C) Sulpiride increased the firing rate of Chls from ChAT Cre animals but not in Chl-Drd2KO cells. Data from individual cells are in gray, with averages overlaid in black for the ChAT Cre and the Chl-Drd2KO in blue. N = 6 ChAT Cre and 8 Chl-Drd2KO mice.
Figure 3. Intact D2 Autoreceptor Function in iMSN and Chl-Drd2KO Mice

(A) Top: Representative DA voltammograms of Drd2loxP/loxP (gray trace) and iMSN-Drd2KO (orange trace) mice. Bottom: Representative traces of evoked DA release in the DLS and corresponding color plots depicting the data with time on the x axis, applied scan potential (Eapp) on the y axis, and background subtracted faradaic current on the z axis in pseudocolor.

(B) Representative traces of DA release under control conditions (no drug application) and after 25 min of 30 nM quinpirole followed by 10–15 mins of 300 nM quinpirole bath application in the Drd2loxP/loxP and iMSN-Drd2KO mice (n = 4 slices/3 mice for each genotypes).

(C) The effects of quinpirole inhibition were similar in both genotypes.

(D) Representative DA traces from DLS and corresponding color plots with DA voltammograms (top) of Drd2loxP/loxP (gray trace) and Chl-Drd2KO (blue trace) mice.

(E) Representative traces (Drd2loxP/loxP and Chl-Drd2KO) of DA transients of the following experimental conditions administered sequentially: (1) after 20 min bath application of DHβE, (2) after 25 mins of 30 nM quinpirole co-applied with DHβE, and (3) after 10–15 min 300 nM quinpirole applied in the presence of DHβE.
(F) The effects of co-application of DHβE and quinpirole was similar in both genotypes (Drd2\textsuperscript{loxp/loxp} n = 6 slices/5 mice; Chl-Drd2KO n = 5 slices/3 mice). All data presented as mean ± SEM.
Figure 4. Loss of LTD in Chl-Drd2KO Mice in Field Potential Recordings

PS amplitudes were normalized to baseline, averaged (mean ± SEM), and plotted as a function of time. Insets: Representative traces before (solid line) and after HFS (dashed line). Scale bars, 0.2 mV, 1 ms. Symbol representation: Drd2^loxP/loxP (gray circles), Chl-Drd2KO mice (blue circles), iMSN-Drd2KO mice (orange circles).

(A) HFS failed to induce LTD in Chl-Drd2KO mice (n = 8 slices/5 mice). However, HFS induced LTD in controls, Drd2^loxP/loxP (n = 6 slices/5 mice).

(B) LTD was blocked during the bath application of AM251 (n = 6 slices/4 mice).

(C) Bath application of sulpiride prevented LTD induction in Drd2^loxP/loxP (n = 5 slices/3 mice).

(D) HFS induced LTD in iMSN-Drd2KO (n = 9 slices/6 mice) and Drd2^loxP/loxP mice (n = 9 slices/5 mice).

(E) HFS-LTD was blocked in the presence of AM251 in both genotypes (Drd2^loxP/loxP n = 5 slices/4 mouse; iMSN-Drd2KO n = 5 slices/3 mice).

(F) HFS-LTD was blocked in the presence of sulpiride (Drd2^loxP/loxP n = 7 slices/6 mice; iMSN-Drd2KO n = 6 slices/5 mice).
Figure 5. LTD Induction at Synapses onto MSNs Is Dependent on Chl D2Rs

The average of the first evoked EPSC amplitudes plotted as a function of time. Insets: Representative traces showing first evoked EPSC before (1) and after (2) LTD induction protocol. Scale bars, 100 pA, 10 ms. Symbol representation: Drd2<sup>loxP/loxP</sup> (gray circles), Chl-Drd2KO mice (blue circles). Scatterplot of individual cell responses (averaged EPSCs during last 10 min of recording) after HFS. Data reported as mean ± SEM.

(A) HFS paired with depolarization induced LTD in Drd2<sup>loxP/loxP</sup> mice (n = 6 cells/6 mice). In contrast, no change in synaptic transmission was observed in the Chl-Drd2KO mice after HFS (n = 7 cells/6 mice).

(B) The magnitude of LTD was variable in Drd2<sup>loxP/loxP</sup> after LTD induction. There was little change in synaptic transmission after HFS in Chl-Drd2KO mice.
(C and D) LTD was lost in both the (C) dMSNs (n = 7 cells/5 mice) and (D) iMSNs (n = 6 cells/4 mice) of the Chl-Drd2KO mice, but it was intact in their littermate controls (dMSN n = 8 cells/7 mice; iMSN n = 7 cells/5 mice).

(E and F) LTD was blocked in the presence of (E) AM251 (n = 7 cells/5 mice) and (F) sulpiride in Drd2\textsuperscript{loxP/loxP} mice (n = 6 cells/5 mice).

(G) Perfusion of quinpirole did not restore LTD in Chl-Drd2KO mice (n = 7 cells/5 mice). HFS in the presence of quinpirole induced LTD in Drd2\textsuperscript{loxP/loxP} mice (8 cells/5 mice).

(H) Bath application of DHPG induced LTD in both genotypes (Drd2\textsuperscript{loxP/loxP} n = 7 cells/6 mice; Chl-Drd2KO n = 6 cells/6 mice).

(I) DHPG-induced LTD was blocked in the presence of AM251 in both genotypes (n = 5 cells/4 mice for each genotype).

(J) HFS in the presence of VU 0255035 restored LTD in the Chl-Drd2KO mice (n = 6 cells/4 mice).

(K) LTD was blocked in the presence of AM251 (n = 5 cells/3 mice).
Figure 6. HFS Can Induce LTD at MSN Synapses in iMSN-Drd2KO Mice

Average evoked EPSC amplitudes plotted as a function of time. Insets: Representative traces showing first evoked EPSC before (1) and after (2) LTD induction protocol. Scale bars, 100 pA, 10 ms. Symbol representation: Drd2\textsuperscript{loxP/loxP} (gray circles), iMSN-Drd2KO mice (orange circles). Scatterplot of individual cell responses (averaged EPSCs during last 10 min of recording) after HFS. Data reported as mean ± SEM.

(A) HFS induced LTD in Drd2\textsuperscript{loxP/loxP} (n = 12 cells/10 mice), but not significantly in the iMSN-Drd2KO mice (n = 7 cells/7 mice).

(B) The magnitude and type of synaptic plasticity varied after HFS in both genotypes.

(C) In the presence of AM251, HFS failed to induce a change in the average EPSC amplitude in both genotypes (n = 5 cells/3 mice for each genotype).

(D) Sulpiride also blocked the induction of LTD in Drd2\textsuperscript{loxP/loxP} (n = 6 cells/6 mice) and iMSN-Drd2KO (n = 5 cells/4 mice) mice.
(E) Pre-application of quinpirole enhanced the magnitude of LTD induction in both genotypes (n = 5 cells/4 mice for each genotypes).

(F) Bath application of DHPG induced LTD in both genotypes (Drd2\textsuperscript{loxP/loxP} n = 5 cells/3 mice; iMSN-Drd2KO n = 6 cells/4 mice).

(G) LTD induction after DHPG bath application was blocked in the presence of AM251 in both genotypes (Drd2\textsuperscript{loxP/loxP} n = 6 cells/5 mice; iMSN-Drd2KO n = 5 cells/4 mice).
Figure 7. LTD Is Lost at Inputs onto iMSNs but not dMSNs in the iMSN-Drd2KO Mice

Insets: Representative traces showing first evoked EPSC before shown in black (1) and after the LTD induction protocol shown in color (2); Drd2<sup>loxP/loxP</sup> EPSC traces (gray), iMSN-Drd2KO traces (orange). The dMSNs are filled color circles; iMSNs are open circles. Scale bars, 100 pA, 10 ms. Data reported as mean ± SEM.

(A) HFS induced LTD in dMSNs from both genotypes (Drd2<sup>loxP/loxP</sup> n = 10 cells/6 mice; iMSN-Drd2KO n = 11 cells/7 mice).

(B) HFS induced LTD in the iMSNs of the Drd2<sup>loxP/loxP</sup> (n = 11 cells/8 mice), but not in the iMSNs of the iMSN-Drd2KO mice (n = 10 cells/7 mice).

(C and D) Quinpirole paired with HFS induced LTD in both (C) dMSNs (n = 6 cells/4 mice for each genotypes) and (D) iMSNs (Drd2<sup>loxP/loxP</sup> n = 5 cells/3 mice; iMSN-Drd2KO n = 8 cells/7 mice) in both genotypes, thus restoring LTD in the iMSNs of the iMSN-Drd2KO mice.