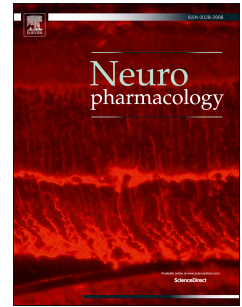


Accepted Manuscript

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PII: S0028-3908(17)30197-1

DOI: [10.1016/j.neuropharm.2017.05.001](https://doi.org/10.1016/j.neuropharm.2017.05.001)

Reference: NP 6703

To appear in: *Neuropharmacology*

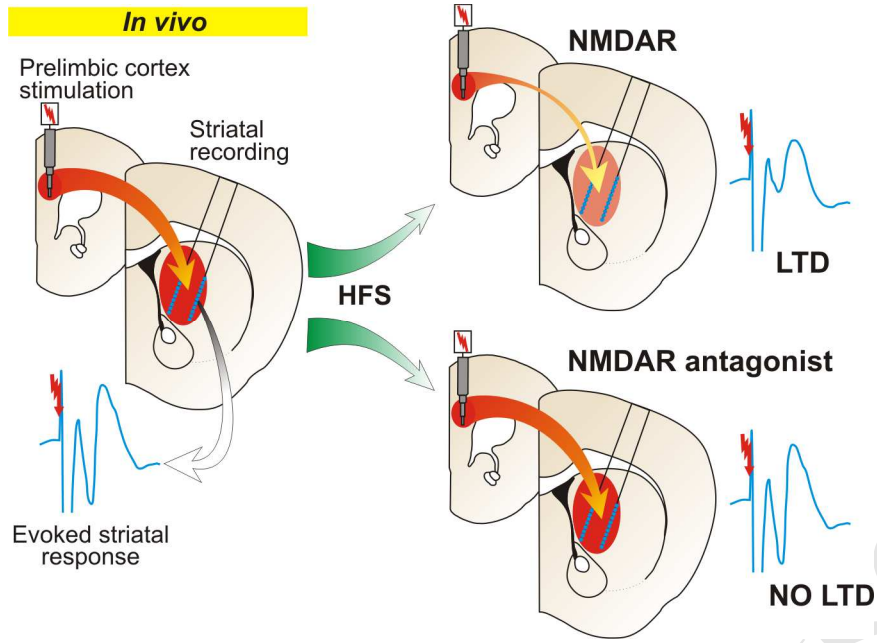
Received Date: 6 January 2017

Revised Date: 24 April 2017

Accepted Date: 1 May 2017

Please cite this article as: Braz, B.Y., Belforte, J., Murer, M.G., Galiñanes, G.L., Properties of the corticostriatal long term depression induced by medial prefrontal cortex high frequency stimulation in vivo, *Neuropharmacology* (2017), doi: 10.1016/j.neuropharm.2017.05.001.

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Properties of the Corticostriatal Long Term Depression induced by Medial Prefrontal Cortex High Frequency Stimulation In Vivo

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Running title: corticostriatal LTD in vivo

ABSTRACT

Repetitive stimulation of cognitive forebrain circuits at frequencies capable of inducing corticostriatal long term plasticity is increasingly being used with therapeutic purposes in patients with neuropsychiatric disorders. However, corticostriatal plasticity is rarely studied in the intact brain. Our aim was to study the mechanisms of corticostriatal long term depression (LTD) induced by high frequency stimulation (HFS) of the medial prefrontal cortex *in vivo*. Our main finding is that the LTD induced in the dorsomedial striatum by medial prefrontal cortex HFS *in vivo* (prefrontostriatal LTD) is not affected by manipulations that block or reduce the LTD induced in the dorsolateral striatum by motor cortex HFS in brain slices, including pharmacological dopamine receptor and CB1 receptor blockade, chronic nigrostriatal dopamine depletion, CB1 receptor genetic deletion and selective striatal cholinergic interneuron (SCIN) ablation. Conversely, like in the hippocampus and other brain areas, prefrontostriatal LTD is NMDA receptor dependent. Thus, we describe a novel form of corticostriatal LTD that operates in brain circuits involved in reward and cognition and could be relevant for understanding the therapeutic effects of deep brain stimulation.

KEYWORDS

long term depression; corticostriatal plasticity; prefrontal cortex; dopamine; endocannabinoids, NMDA receptor; striatum

1. INTRODUCTION

The striatum is the main input nucleus of the basal ganglia and receives topographically ordered inputs from most cortical areas (Hintiryan et al. 2016; Voorn et al. 2004). Projections from prefrontal associative areas are directed mainly to the medial and ventral striatum while those from motor cortical areas reach the dorsolateral striatum. This organization of cortical input relates to functional differences between ventromedial and dorsolateral striatum where the former contributes to more flexible forms of behavior than the latter, which is involved in habits (Everitt & Robbins 2005; Yin & Knowlton 2006; Graybiel 2008). It has been proposed that long term changes in corticostriatal transmission underlie the acquisition of goal directed behaviors and habits (Hawes et al. 2015; Reynolds et al. 2001; Costa et al. 2004). The best described form of corticostriatal synaptic plasticity is long term depression (LTD) induced by high frequency stimulation (HFS) of striatal afferents. While corticostriatal LTD can be induced by HFS both *ex vivo* (P Calabresi et al. 1992; Lovinger et al. 1993; Walsh 1993) and *in vivo* (Reynolds & Wickens 2000), its molecular and pharmacological characterization has mainly been done in brain slices.

Ex vivo studies focused in the dorsolateral striatum show that HFS-LTD depends on dopamine (DA) D2 receptor stimulation (David M Lovinger 2010) and postsynaptic endocannabinoid (eCB) release leading to presynaptic CB1 receptor activation and reduced probability of glutamate release (Choi & Lovinger 1997b). Since HFS-LTD is expressed in striatal medium spiny projection neurons (MSNs) of both the indirect (iMSN) and direct (dMSN) pathways, but DA D2 receptors are only expressed in iMSN, it has been proposed that DA dependent forms of LTD are mediated by striatal cholinergic interneurons (SCIN), which express high levels of D2 receptors and regulate both dMSNs and iMSNs (Wang et al. 2006). A few *ex vivo* studies addressing synaptic plasticity in the dorsomedial and ventral striatum revealed regional differences in corticostriatal plasticity (Partridge et al. 2000; Thomas et al. 2000; Dang et al. 2006). For instance, LTD is regarded as NMDA receptor independent in the dorsolateral striatum but it requires NMDA receptors in the ventral striatum (Thomas et al. 2000). The mechanisms of corticostriatal LTD have rarely been addressed with *in vivo* approaches (Reynolds & Wickens 2000). Whether corticostriatal HFS-LTD requires DA, eCBs, SCIN and NMDA receptors *in vivo* remains to be clarified.

In a previous *in vivo* study we concluded that the local field potential response evoked in the dorsomedial striatum (eLFP) by electrical stimulation of the medial prefrontal cortex (mPFC) of mice reflects cortical input to MSNs based on the following observations: i. its amplitude is linearly correlated to the amplitude of depolarizing postsynaptic potentials simultaneously recorded from MSNs; ii. it is blocked by intrastriatal infusion of an AMPA receptor antagonist; iii. its topographical organization is consistent with the anatomical projections of the mPFC to the dorsal striatum (Galiñanes et al. 2011). In the present study we performed striatal eLFP recordings in mice under urethane anesthesia to analyze the properties of the corticostriatal LTD induced by HFS of the mPFC. We found that this form of LTD is DA and CB1 receptor independent and is unaffected by selective SCIN ablation. Interestingly, we also found that prefrontostriatal HFS-LTD is NMDA receptor dependent.

2. METHODS

2.1 Animals

Mice were maintained under a 12 h light:12 h dark cycle with ad libitum access to food and water and cared for in accordance with institutional (IACUC, RS2964/2010 and 2598/13, University of Buenos Aires) and government regulations (SENASA, RS617/2002, Argentina). All efforts were made to minimize the number of animals used and their suffering.

In all pharmacological experiments we used adult CF-1 mice (12-30 weeks old). For SCIN lesion experiments we used ChAT-Cre^{+/-};DTR^{loxP/wt} mice (ChAT-DTR mice) in C57BL/6 homogeneous background (Martos et al. 2017). ChAT-DTR mice were generated by crossing homozygous ChAT-Cre mice (Chat^{tm(cre)Lowl/J}, Jackson Laboratories, J06410) with homozygous iDTR mice (Gt(ROSA)26Sor^{tm1(HBEGF)Awai}, Jackson Laboratories, J007900). The iDTR line allows expression of the Diphtheria Toxin Receptor (simian Hbegf) after Cre-mediated recombination of a floxed stop cassette, rendering targeted cells susceptible to the toxin. CB1 receptor knock-out mice (CB1 KO) were generated by crossing homozygous CB1 KO mice in CD1 background with wild type CD1 mice (Wolfson et al. 2015).

2.2 Lesions and pharmacology

Neonatal dopamine neuron lesions were done in CF-1 mice as previously described (Avale et al. 2004). PD2 pups received bilateral injections of the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA, 6.25 $\mu\text{g}/\mu\text{l}$; MP Biomedicals) or vehicle (0.1% ascorbic acid) in each lateral ventricle (1.1 mm below the skin, 0.6 mm from midline, and 1.5 mm anterior to the lambda), after desipramine pretreatment (20 mg/kg, s.c.) to protect noradrenergic neurons, under hypothermal anesthesia. Injections were performed at a constant rate of 1.25 $\mu\text{l}/\text{min}$ with a 30 gauge needle coupled to a 25 μl Hamilton syringe driven by a microinfusion pump (Bee syringe pump and controller, Bioanalytical Systems). In each litter, half of the pups received the toxin and the other half vehicle. After surgery, they were warmed up and returned to their home cages in groups of up to eight pups per breast-feeding mother until weaning (PD24). Thereafter, control and lesion mice were housed together in the same cage in groups of 4-6 until the electrophysiological experiment (12-30 weeks).

SCIN lesions were performed in ChAT-DTR mice (3-4 month old) as described by (Martos et al. 2017). Under deep surgical anesthesia (isofluorane 1-2%) each mouse was mounted in a stereotaxic frame (Stoelting Co, USA) with a mouse-adaptor and treated with a local anesthetic in the scalp and pressure points (bupivacaine hydrochlorate solution, 5% wv/v, Durocaine, AstraZeneca S.A., Argentina 0.1-0.3 ml). Ophthalmic ointment was applied in both eyes to prevent corneal desiccation. Diphtheria Toxin stocks (DT, Sigma #D0564) were freshly diluted in sterile saline to a concentration of 200pg/ μl . DT solution (lesion) or saline (sham) were microinjected (at a constant rate of 0.22 $\mu\text{l}/\text{min}$) unilaterally in dorsal striatum via a 30 gauge stainless steel cannula coupled to a 10 μl Hamilton syringe driven by a microinfusion pump (Bioanalytical Systems, USA). The injection cannula was left in place for one additional minute before slowly retracting it. DT solution or solvent were injected in three independent sites per hemi-striatum: anterior site: 1.3 mm anterior to bregma, 1.6mm lateral and -2.8mm (0.8 μl) and -2.4mm (0.8 μl) ventral from dura; posterior site: 0.6mm anterior to bregma, 1.8mm lateral and -3mm ventral from dura (1.2 μl) according to the atlas of Paxinos and Franklin (2001). Control and lesion mice were housed together in groups of 3-5 animals per cage until testing.

Chemicals were purchased from Sigma or Tocris. The CB1 receptor antagonist AM-251 was dissolved in DMSO 0.5% and administered at a dose of 5 mg/kg two hours before HFS. DA

receptor antagonists eticlopride and SCH23390 were dissolved in saline and co-administered at a dose of 0.25 mg/kg, 90 minutes before HFS. MK-801, an NMDA receptor antagonist, was dissolved in saline and administered in two 0.375 mg/kg i.p. injections separated by 15 minutes (final dose: 0.75 mg/kg) due to the lethality observed after delivering the total dose in one injection.

2.3 Electrophysiology

All recordings were performed under urethane anesthesia (1.2–1.5 g/kg i.p.) following published protocols (Galiñanes et al., 2009, 2011). Bupivacaine was applied subcutaneously on the scalp and pressure points and the animal was affixed to a stereotaxic frame. Body temperature was maintained at 36–37 °C with a servocontrolled heating pad (Fine Science Tools, Vancouver, Canada). During the experiment, the level of anesthesia was regularly verified by testing the nociceptive hind limb withdrawal reflex and by online visual examination of the frontal cortex electrocorticogram (see below). Supplemental doses of urethane were customarily given throughout the experiment (0.3 g/kg s.c. every 2–3 h).

A concentric bipolar stimulating electrode (SNE-100, Better Hospital Equipment, New York, NY; outer contact diameter 0.25 mm, central contact diameter 0.1 mm, contacts separation 0.75 mm, contact exposure 0.25 mm) was placed into the prelimbic area of the mPFC (2.0 mm anterior to bregma, 0.4 lateral to midline, 2.0 mm ventral to the cortical surface, ipsilateral to the striatal recording site, Paxinos and Franklin, 2001). A second bipolar electrode was placed into the motor cortex (2.0 mm anterior to bregma, 1.6 lateral, 1.2 mm ventral) to record the electrocorticographic activity (0.1–300 Hz (Galiñanes et al. 2009; Galiñanes et al. 2011)). Striatal field potentials were recorded with a 24-channel two-shank silicon probe (100 µm vertical spacing between contacts and 500 µm horizontal shank spacing; NeuroNexus Technologies, Ann Arbor, MI). Each recording site of the silicon probe had a contact area of 413 µm² and an impedance of about 0.8 MΩ. The probe was positioned in the coronal plane within the rostral area of the dorsal striatum with an angle of 20° from the vertical midline (0.6 mm anterior to bregma, 2.5 mm lateral to midline, 3.2 mm ventral to the cortical surface). The signals were referenced to a screw in the interparietal bone, amplified, band-pass filtered to obtain local field potential (5–300 Hz) and multiunitary action potential (300–3000 Hz) activities proceeding from each recording site, digitized (10 kHz) and stored in a computer for offline analysis.

Constant current pulses (50 pulses, 0.3 ms duration at 0.1 Hz, 350 μ A; Iso-Flex and Master 8, AMPI, Jerusalem, Israel) were applied to study the striatal eLFP and multiunitary action potential responses to mPFC stimulation. After acquisition of baseline responses HFS was delivered through the same mPFC electrode at 750 μ A (four trains: 1 s duration, 100 Hz frequency, 10 s interval), and then, test responses to mPFC stimulation were recorded for at least 2 hs. The eLFP was analyzed off line with custom Matlab routines. Electrical stimulation of the mPFC evoked a complex striatal field potential response typically consisting of a positive-negative-positive (P1-N2-P2) waveform (Figure 1A-B; Galiñanes et al., 2011). The amplitude of the eLFP was determined as the voltage difference between the N2 peak and the subsequent P2 peak, which correlates with the amplitude of intracellularly recorded EPSPs from MSNs and is stable for at least two hours of recording (Galiñanes et al., 2011). For every individual trial the voltage and latency of the N2 and P2 peaks were semiautomatically determined by detecting the local minimum and maximum of the eLFP. To quantify the action potential response, the signal was rectified, smoothed and averaged, allowing the computation of the area under the curve and peak latency of the multiunitary action potential response (Galiñanes et al., 2011).

2.4 Histology and immunohistochemistry

At the end of each experiment mice received a lethal dose of urethane and were transcardially perfused with 10 ml of 0.04 % heparine cold saline solution (Sodic Heparine, Duncan Laboratories, 5000 UI/ml) followed by 20 ml of paraformaldehyde (PFA, 4%) in 0.1 M PBS. Brains were removed, immersed over night in the same fixative at room temperature, and stored in 0.1 M PBS containing 30% sucrose at 4°C for 24–72 h. Thirty μ m thick coronal brain sections were cut in a freezing microtome (Leica, USA).

Dopamine neuron depletion was confirmed by immunohistochemical detection of tyrosine hydroxylase (TH) as described before (Galiñanes et al. 2009). Sections were successively incubated with rabbit anti-TH antibodies (1:1000, Chemicon), anti-rabbit biotinylated antibodies (1:250, Vector), streptavidin-biotin/horseradish peroxidase complex (1:125, ABC kit Vector Laboratories, USA), and reacted with 3,3'-diaminobenzidine (DAB, Sigma USA).

The extent of SCIN lesion was confirmed by immunohistochemical detection of ChAT. Briefly, sections were incubated in goat anti-ChAT antibody (1:1000, AB 144P Millipore, USA), then with

a horse anti-goat biotinylated secondary antibody (1:250, BA-9500, Vector Labs, USA) and subsequently with streptavidin-biotin/horseradish peroxidase complex and DAB. Lesions were assessed by counting ChAT+ cell bodies in the dorsal striatum. Sections were photographed and digital files analyzed using ImageJ cell counter plugin. All immunoreactive cell bodies with at least one stained process were counted in the dorsal striatum of the lesioned hemisphere in two sections per mouse (coronal planes +1.2/1.3, +0.4/0.6 mm from bregma). Electrophysiological experiments were done three to eight weeks after the surgery. In this period of time no further reduction in SCIN number occurred (Martos et al. 2017).

Location of the cortical stimulation electrode was assessed by visual examination of the mechanical tissue damage in the coronal sections using a transmitted light microscope at low magnification. In order to determine the location of the striatal recording sites, before each electrophysiological experiment the multi-electrode was immersed in the red fluorescent dye 1,19-dioctadecyl-3,3,39,39-tetramethylindocarbocyanine perchlorate (100 mg/ml in acetone; Dil, Molecular Probes) and air dried for 30 minutes before use. This allowed post mortem detection of the fluorescent material deposited in the tissue with an epifluorescence microscope.

2.5 Statistical analysis

Data were organized using Microsoft Excel and analyzed using SigmaPlot 11.0. One-way or two-way repeated measures ANOVA followed by Holm-Sidak or Tukey post-hoc test were employed, as described in the figure legends. For normalized data we only included the test period in the statistical analysis. Data are presented as mean \pm SEM if not indicated otherwise.

3. RESULTS

3.1 High frequency stimulation of the prefrontal cortex induces a sustained depression of corticostriatal transmission

Previous *in vivo* studies have shown that different protocols of stimulation induce corticostriatal LTD in non-anesthetized (Stoetzner et al. 2010) and urethane-anesthetized (Reynolds & Wickens 2000) animals, but not in animals anesthetized with barbiturates, in which both low and high frequency stimulation protocols induce LTP (Charpier & Deniau 1997; Charpier et al. 1999). Thus, we studied the effect of delivering HFS to the mPFC on the response evoked in the striatum by mPFC stimulation under urethane anesthesia (figure 1A-C). The intensity of test and HFS stimuli were set at 350 μ A and 750 μ A respectively, which according to previous studies induce half maximal and maximal striatal responses to mPFC stimulation respectively (Galiñanes et al. 2011; Braz et al. 2015). HFS consisted of four 1s-duration 100 Hz stimulation trains of stimuli, as in most *ex vivo* studies (Figure 1C). Relative to baseline, HFS produced a long lasting reduction of the striatal eLFP amplitude ($30\pm 6\%$ decrease 100 min after HFS; figure 1C) and of the simultaneously recorded multiunitary action potential response ($44\pm 6\%$ decrease; figure 1D), consistently with *ex vivo* studies (P Calabresi et al. 1992; Lovinger et al. 1993; Walsh 1993). In a previous *in vivo* study we showed that the amplitudes of the eLFP and multiunitary action potential response are linearly correlated to each other (Galiñanes et al. 2011). We observed that after HFS this relationship is maintained. There was a strong correlation between the normalized eLFP and action potential area after HFS (figure 1F; Pearson correlation: $R=0.7$, $p<0.001$; 12 animals, 7 ± 1 channels/animal). Thus, the data show that mPFC HFS produces a sustained reduction of corticostriatal transmission.

Next, we analyzed the effect of HFS on the "paired pulse ratio" (PPR), which is extensively studied *ex vivo* as a tool for interpreting presynaptic changes associated with long term plasticity. Test stimuli were delivered in pairs separated by 50 ms at 0.2 Hz. Although there was a transient increase in PPR after HFS (figure 1F), consistently with *ex vivo* studies relating LTD to a reduced probability of glutamate release (Gerdeman et al. 2002), PPR returned to baseline values 50 min after HFS. Since a connection between changes in PPR and neurotransmitter

release probability has not been established for *in vivo* conditions, we did not study PPR in subsequent experiments.

3.2 Prefrontostriatal HFS-LTD is dopamine receptor independent

Ex vivo studies focused in the dorsolateral striatum show that DA is necessary for corticostriatal HFS-LTD, especially through D2 DA receptor activation (P. Calabresi et al. 1992; Tang et al. 2001; Wang et al. 2006). However, Reynolds and Wickens (2000) reported that, *in vivo*, corticostriatal HFS-LTD is preserved in the lateral striatum of animals in which catecholamine synthesis was acutely inhibited by α -methyl-para-tyrosine and is prevented by concurrent electrical stimulation of the substantia nigra. Here we studied prefrontostriatal HFS-LTD in animals that received an acute challenge with D1 and D2 DA receptor antagonists at doses that produce catalepsy in awake mice (Eticlopride 0.25 mg/kg, SCH23390 0.25 mg/kg, i.p.). We found that concomitant administration of D1 and D2 DA receptor antagonists does not block prefrontostriatal HFS-LTD *in vivo* (figure 2A). Furthermore, chronic dopamine neuron lesions (6-OHDA i.c.v. injection at PD2), which have been shown to abolish corticostriatal HFS-LTD in the dorsolateral striatum in brain slices (Tang et al. 2001), did not impair prefrontostriatal HFS-LTD *in vivo* (figure 2B). Thus, DA seems to not be necessary for HFS-LTD expression in the prefrontostriatal circuit.

3.3 Prefrontostriatal HFS-LTD is CB1 receptor independent

Postsynaptic eCB release from MSN has been described in *ex vivo* studies as a key mechanism for HFS-LTD that leads to presynaptic CB1 receptor activation and a reduction of glutamate release (Ronesi et al. 2004). However, CB1 receptor expression is higher in the lateral striatal area where most *ex vivo* studies are focused than in the medial striatum where mPFC afferents preferentially end (Hohmann & Herkenham 2000), and decreases markedly with age (Zhang et al. 2015). Moreover, some recent *ex vivo* studies describe forms of corticostriatal LTD that seem to be CB1 receptor independent (Mathur et al. 2011; Atwood et al. 2014; Rafalovich et al. 2015). To assess if CB1 receptor activation is necessary for prefrontostriatal HFS-LTD *in vivo* we performed experiments in adult mice acutely treated with the CB1 receptor antagonist AM-251 (5 mg/kg in DMSO 0.5%, i.p.) and in CB1 receptor knock-out mice. HFS-LTD could be induced in

both conditions (figure 3) suggesting that CB1 receptor activity is not necessary for prefrontostriatal HFS-LTD *in vivo*.

3.4 Prefrontostriatal HFS-LTD is preserved in striatal cholinergic interneuron depleted mice

SCIN have been involved in DA dependent forms of LTD at corticostriatal synapses of the dorsolateral striatum (Wang et al. 2006). To investigate if prefrontostriatal HFS-LTD also depends on SCIN we recorded from animals with unilateral SCIN depletion performed with a diphtheria toxin (DT) inducible cell ablation system (Martos et al. 2017). To direct selective DT receptor (DTR) expression into cholinergic cells, we crossed the transgenic lines ChAT-Cre and iDTR (which has the human DTR sequence flanked by loxP sites) to generate the offspring ChAT-Cre^{+/-}; DTR^{loxP/wt} (hereafter ChAT-DTR). Injection of the DT in the striatum of ChAT-DTR mice produced a marked loss of cholinergic striatal interneurons. The control group consisted of ChAT-DTR mice injected with saline (n=3) and DTR^{loxP/loxP} mice injected with DT (n=2). Intra-striatal DT injections induced a 95% SCIN loss in ChAT-DTR mice that extended throughout the striatum (figure 4A-B). HFS-LTD was conserved in SCIN depleted animals (figure 4C), suggesting that SCIN are not necessary for prefrontostriatal HFS-LTD *in vivo*.

3.5 Corticostriatal HFS-LTD is NMDA receptor dependent

Corticostriatal HFS-LTD is NMDA receptor independent in the dorsolateral striatum *ex vivo* (P Calabresi et al. 1992; Walsh 1993; Partridge et al. 2000). However, certain forms of LTD depend on or are regulated by NMDA receptors in the hippocampus and other structures (Carter & Jahr 2016; Thiels et al. 1996; Pontrello et al. 2012; Sidorov et al. 2015), including the nucleus accumbens (Thomas et al. 2000). To determine if NMDA receptors are necessary for prefrontostriatal HFS-LTD we performed experiments in animals that received acute injections of the NMDA receptor antagonist MK-801 (two 0.375 mg/kg i.p. injections separated by 15 minutes). By itself, administration of MK-801 produced a reduction of the eLFP amplitude (5±3% reduction 15 minutes after the first injection and 14±4% reduction 15 min after the second injection, relative to baseline; figure S1). HFS application 15 min after the second injection of MK-801 did not produce any additional reduction of the eLFP amplitude (figure 5 and figure S1), suggesting a role of NMDA receptors in the mechanism of prefrontostriatal HFS-LTD.

To rule out a floor effect after the corticostriatal depression caused by MK-801, we tested in naive mice whether HFS can depress an eLFP that has already been depressed by a previous round of HFS. In the absence of MK-801 we applied the regular protocol of HFS-LTD and at the end of the test period we applied a second round of HFS. As a result, eLFP amplitude was depressed an additional $19\pm 9\%$ (total depression at the last block: $45\pm 5\%$; figure S2) indicating that the absence of HFS-LTD under the effect of MK-801 is not due to a floor effect.

In sum, these results show that prefrontostriatal HFS-LTD is NMDA receptor dependent.

4. Discussion

We found that HFS of the mPFC consistently depresses the striatal response to mPFC stimulation *in vivo* through a mechanism that withstands conditions that would block the most common form of LTD observed in the dorsolateral striatum in brain slices, including DA receptor and CB1 receptor blockade, chronic DA depletion, CB1 receptor deletion, and SCIN ablation. In addition, while LTD is regarded as NMDA receptor independent in the dorsolateral striatum, it could not be induced in the dorsomedial striatum after the administration of an NMDA receptor antagonist. Thus, prefrontostriatal LTD bears more resemblance to NMDA receptor dependent forms of LTD described in the hippocampus and the nucleus accumbens than to the canonical form of LTD that operates in the dorsolateral striatum.

Dopamine is thought to shape behavior through its effects on synaptic plasticity (Hikosaka 2014; Berridge 2013). DA participation in LTD at the lateral striatum through D2 receptor activation has been replicated in brain slice studies using different induction paradigms like HFS and spike timing dependent plasticity (STDP) (Tang et al. 2001; Kreitzer & Malenka 2005; Wang et al. 2006; Shen et al. 2008; P Calabresi et al. 1992); (but see Pawlak & Kerr 2008). Not only DA receptor antagonists but also deletion of D2 receptors and chronic DA lesions disrupt HFS-LTD in the lateral striatum (P Calabresi et al. 1992; Kreitzer & Malenka 2005; Tang et al. 2001). However, LTD can be induced *ex vivo* in the presence of D2 receptor antagonists by the application of mGluR1 agonists (Kreitzer & Malenka 2005) or by the activation of L-type calcium channels in combination with afferent stimulation (Adermark & Lovinger 2007), suggesting that DA is modulatory rather than strictly necessary for LTD (David M. Lovinger 2010; Pawlak et al.

2010). Moreover, a previous *in vivo* study has shown that HFS-LTD is preserved in the lateral striatum after acute inhibition of catecholamine synthesis (Reynolds and Wickens, 2000).

While the sources of DA during HFS may differ between the *in vivo* and slice preparations, it is unclear whether these differences are responsible for the lack of DA dependency of HFS-LTD in our study. *In vivo*, a striatal dopaminergic tone is maintained by the spontaneous tonic firing of nigrostriatal dopaminergic neurons (Kelland et al. 1990; Tepper et al. 1991). Moreover, in the intact brain long range connections like the excitatory projections connecting the mPFC to the ventral tegmental area can induce phasic DA release in the striatum during cortical electrical stimulation (Murase et al. 1993). In coronal brain slices, where dopaminergic projections are cut, it is not clear whether a dopaminergic tone is spontaneously maintained (Wu et al. 2015), but HFS in or near the striatum directly stimulates dopaminergic fibers and DA release, in addition to stimulating cortical and thalamic afferents to MSNs (Kreitzer & Malenka 2008). In our study the intracortical location of the stimulating electrode makes less likely a direct activation of dopaminergic and thalamic terminals near the recording site. Additional mechanisms may contribute to DA release both *in vivo* and *ex vivo*. For instance, cortical input to the dorsal striatum locally regulates striatal DA release via activation of ionotropic glutamate receptors on SCIN which, in turn, activate nAChRs on dopaminergic axons to trigger DA release (Kosillo et al. 2016). In turn, DA inhibits ACh release by SCIN, relieving MSN from the negative modulation exerted by muscarinic receptors on L-type calcium channels, and rescuing HFS-LTD from D2 receptor antagonists in brain slices (Wang et al. 2006). However, SCIN ablation did not block LTD *in vivo*.

Among the more prominent factors that may contribute to the resilience of HFS-LTD to DA and ACh restriction *in vivo* is spontaneous activity, which is higher in the intact circuit than in coronal brain slices. In rodents, even under anesthesia, cortical neurons and striatal MSNs display robust depolarizations (Up states) during which they are enabled to fire action potentials (Mahon et al. 2001; Kasanetz et al. 2002; Tseng et al. 2001; Galiñanes et al. 2011). *In vivo* the Up states are sustained by glutamate released from cortical (and thalamic) afferents and they are absent in MSNs when these afferents are disconnected or silent (Wilson 1993; O'Donnell & Grace 1993; Kasanetz et al. 2006). Up states are also absent or very infrequent in brain slices where cortical neurons do not show spontaneous Up states at the concentrations of

divalent cations used in standard artificial cerebrospinal fluid (Castro-Alamancos & Favero 2015; Cunningham et al. 2006). Thus, in the slice preparation, MSNs remain at a very polarized resting potential known as Down state (reviewed by Murer and O'Donnell, 2016). Importantly, MSNs need to be depolarized as if to simulate an Up state during HFS to obtain LTD in brain slices (Kreitzer & Malenka 2008). This depolarization is intended to activate L-type calcium channels and to increase intracellular calcium, which is required for LTD induction (Calabresi et al. 1994; Choi & Lovinger 1997a). Relieving L-type calcium channels from the negative modulation exerted by D2 or M1 receptors (Olson et al. 2005) may be necessary to obtain enough calcium entry into MSN during LTD induction in brain slices, but L-type channel activation may be facilitated *in vivo* by the higher levels of spontaneous activity (Calabresi et al. 1994; Choi & Lovinger 1997a), making LTD resistant to DA and ACh restriction. Moreover, activation of NMDA receptors during Up states (Pomata et al. 2008) provides a complementary source of intracellular calcium which may turn MSN plasticity less dependent on levels of neuromodulators.

Ex vivo studies focused in the dorsolateral striatum consistently involve activity-dependent eCB release by postsynaptic cells through calcium-dependent or G-protein-coupled receptor (GPCR)-dependent processes in corticostriatal LTD (Heifets & Castillo 2009; David M Lovinger 2010; Zlebnik & Cheer 2016). However, additional mechanisms of LTD have been described in brain slice studies of the striatum that do not depend on eCB. For instance, serotonin (Mathur et al. 2011), opioids (Atwood et al. 2014) and nitric oxide (Rafalovich et al. 2015) induce LTD through CB1 receptor independent mechanisms. Endocannabinoid-independent mechanisms of LTD could be less prevalent in the medial striatum because the expression of CB1 receptors is lower there than in the lateral striatum (Hohmann & Herkenham 2000). Interestingly, in the dorsomedial striatum LTP prevails over LTD for a longer period of postnatal development than in the dorsolateral striatum (Partridge et al. 2000). Further studies support the existence of differences between the forms of long term plasticity operating in the dorsolateral and dorsomedial striatum (Atwood et al. 2014; Yin et al. 2007).

An intriguing finding is the blockade of HFS-LTD by the NMDA receptor antagonist MK-801. The involvement of the NMDA receptor in LTD is common in other structures like the hippocampus, but has not been reported in the dorsal striatum. In the hippocampus, low frequency

stimulation patterns and spike timing protocols where postsynaptic activity precedes presynaptic activity, induce LTD, while high frequencies of stimulation and protocols where presynaptic spikes precede postsynaptic spikes, induce LTP (Kemp et al. 2000; Raymond 2007). Moderate activation of NMDA receptors with the former protocols leads to LTD whereas stronger activation with the later produces LTP (Luscher & Malenka 2012). Importantly, in the nucleus accumbens, low and moderate frequency stimulation of glutamatergic afferents induces a NMDA receptor-dependent form of LTD (Thomas et al. 2000; Dang et al. 2006). Thus, the LTD we observe *in vivo* in the dorsomedial striatum may resemble more some LTD forms that operate in the ventral striatum and other structures than the canonical eCB-dependent LTD that operates in the dorsolateral striatum.

In summary, the results presented here highlight the importance of studying corticostriatal plasticity with *in vivo* preparations. Also, they stress that dorsomedial and dorsolateral striatum may be subject to different plasticity rules contributing to their differential functional role in behavior. The study of the *in vivo* consequences of repetitive stimulation of central circuits has become clinically relevant because deep brain stimulation and repetitive transcranial magnetic stimulation are increasingly used to treat conditions like obsessive compulsive disorder and Parkinson's disease. Further studies are needed to understand the long term consequences of such interventions in the normal and diseased brain.

Acknowledgements

We thank Graciela Ortega and M. Brahamian for excellent technical assistance. This work was supported by grants from FONCYT (PICT 2011-0521, MGM and JEB; 2013-1523, MGM; 2015-3687, MGM), University of Buenos Aires (UBACYT-2014, MGM) and Tourette Association of America (JEB and MGM).

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Figures

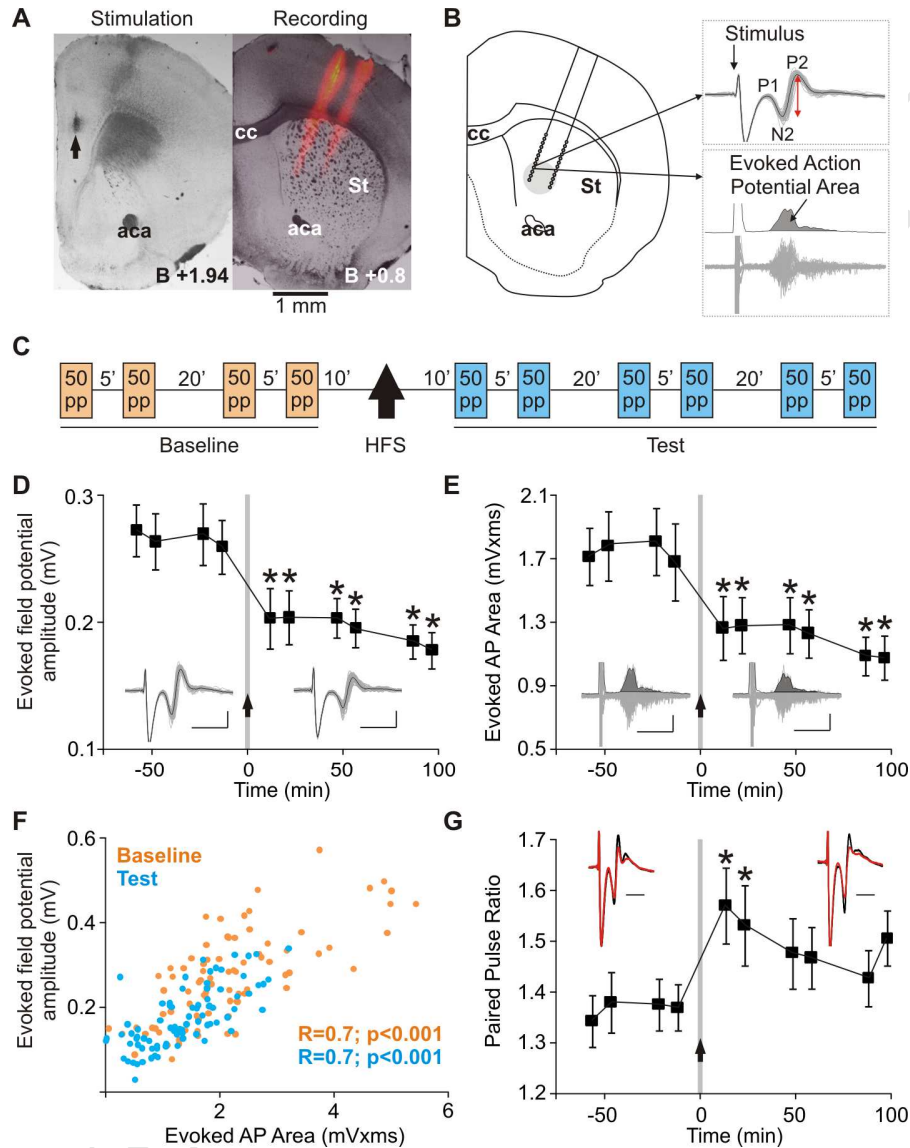


Figure 1: Corticostriatal long term depression *in vivo*. **A.** Representative histological sections showing the location of cortical stimulation electrode in the mPFC (arrow) and Dil deposit (red fluorescence) indicating the location of the silicon probe in the striatum (aca: anterior commissure; St, striatum; cc: corpus callosum; B+: anterior to Bregma). **B.** Reconstruction of the 24 striatal recording sites from the example shown in A and representative traces (grey) and average (black) of eLFP and action potential responses evoked by cortical stimulation. Channels located in the grey shaded area, which showed the strongest response to mPFC stimulation

(Galiñanes et al., 2011), were averaged. Field potential amplitude was measured between the N2 and P2 peaks (upper panel). To quantify the multiunitary action potential response (lower panel), trials were rectified, smoothed and averaged. **C.** Protocol used to assess HFS-LTD *in vivo*. We tested the striatal response to mPFC stimulation before (baseline, orange) and after (test, blue) HFS. Each orange or blue square represents a block of 50 paired pulses delivered at 0.2 Hz (pulse duration: 0.3 ms, pulse intensity: 350 μ A, inter-stimulus interval: 50 ms). **D-E.** HFS was applied at time 0 (arrow). Example traces for the first and the last blocks are shown (individual traces in grey and average in black). Scale: 0.1 mV (eLFP), 1 mVxms (AP area), 10 ms. One way RM-ANOVA, significant effect of time, * $p < 0.05$ Holm-Sidak comparisons. **F.** Correlation between the eLFP and action potential response during the last block of the baseline (orange) and the test (blue) periods (Pearson correlation, $R = 0.7$, $p < 0.001$ for both sets of data). Each point corresponds to an individual channel (12 animals, 7 ± 1 channels/animal). **G.** Paired pulse ratio showed a transient increase after HFS application. Representative eLFP averages for the first (red) and second pulse (black) are shown for baseline and the first block of the test period (scale 10 ms). One way RM-ANOVA, significant effect of time, * $p < 0.05$ Holm-Sidak comparisons.

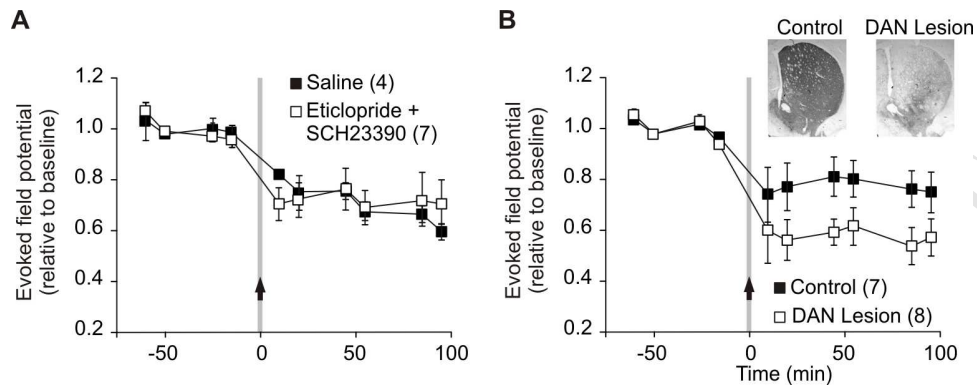


Figure 2: HFS-LTD *in vivo* is DA independent. **A.** Systemic administration of DA receptor antagonists (Eticlopride 0.25 mg/kg, SCH23390 0.25 mg/kg, i.p., 90 minutes before HFS) had no effect on HFS-LTD (two way RM-ANOVA, no significant interaction, treatment factor $p=0.9$). **B.** HFS-LTD is not occluded by neonatal dopamine neuron depletion (two way RM-ANOVA, no significant interaction, treatment factor $p=0.08$). Top: coronal sections of two representative mice immunostained for TH showing the loss of nigrostriatal axons in the striatum of the mouse with dopamine neuron lesion (DAN).

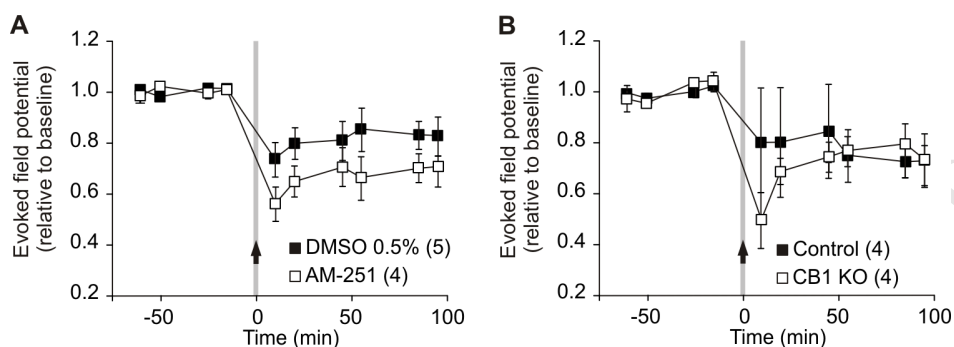


Figure 3. HFS-LTD in vivo is CB1 receptor independent. **A.** Systemic administration of the CB1 receptor antagonist AM-251 (DMSO 0.5%, 5 mg/kg i.p., two hours before HFS) did not block HFS-LTD (two way RM-ANOVA, no significant interaction, treatment factor $p = 0.2$). **B.** CB1 receptor knock-out mice show HFS-LTD (two way RM-ANOVA, no significant interaction, treatment factor $p = 0.6$).

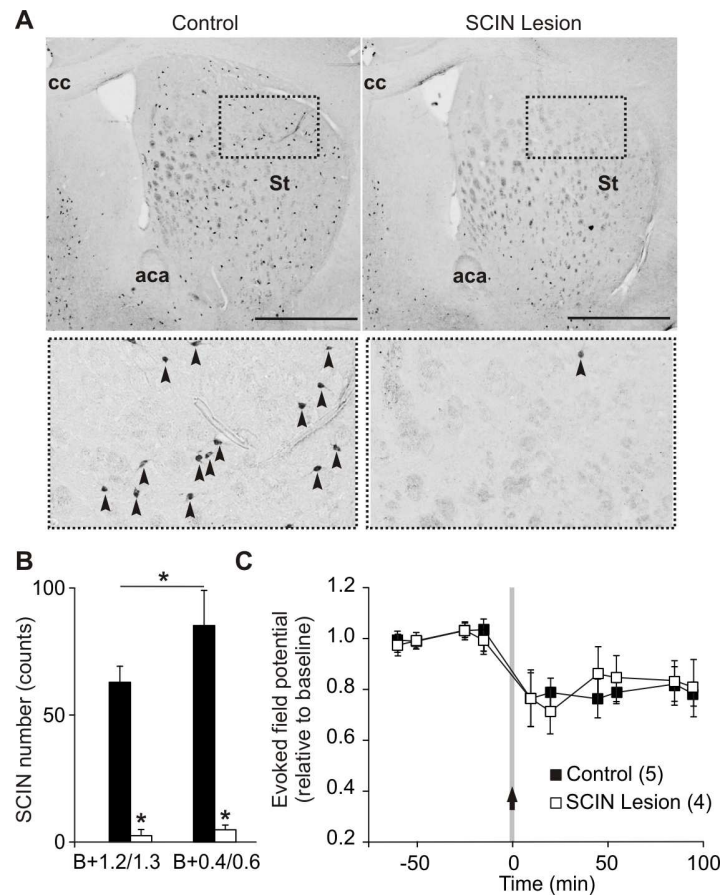


Figure 4. HFS-LTD in animals depleted of SCIN. A. Striatal sections immunostained for ChAT in representative control and SCIN-lesioned mice (aca: anterior commissure; St: striatum; cc: corpus callosum; scale= 1 mm). SCIN lesion was performed unilaterally by intrastriatal diphtheria toxin (DT) injections in transgenic mice expressing the human DTR in ChAT positive neurons. Lower panel: Arrows point to ChAT positive neurons (lower panel). **B.** Quantification of SCIN loss at two coronal levels (B+: anterior to Bregma). Two way RM-ANOVA, * $p < 0.05$ Tukey post hoc comparisons after significant interaction. **C.** SCIN lesion had no effect on HFS-LTD expression *in vivo* (two way RM-ANOVA, no significant interaction, treatment factor $p = 0.9$).

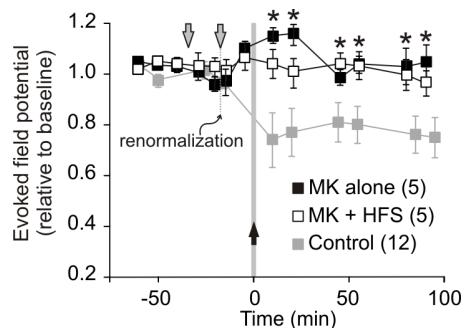


Figure 5. HFS-LTD is NMDA receptor dependent. Application of MK-801 (grey arrows, 0.75 mg/kg i.p., two injections at grey arrows) produces a slight reduction of the basal eLFP amplitude. After HFS application (black arrow) there was no further depression of the eLFP in mice treated with MK-801. Two way RM-ANOVA, no significant interaction, significant effect of treatment, * $p < 0.05$ Tukey comparisons, control versus MK-801 alone and control versus MK-801 + HFS.

HIGHLIGHTS

- Medial prefrontal cortex HFS induces corticostriatal LTD *in vivo*
- Prefrontostriatal HFS-LTD is dopamine and CB1 receptor independent *in vivo*
- *In vivo*, prefrontostriatal HFS-LTD does not require striatal cholinergic interneurons
- Pharmacological NMDA receptor blockade blocks prefrontostriatal HFS-LTD *in vivo*