Induction frequency affects cortico-striatal synaptic plasticity with implications for frequency filtering

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Abstract

Long-term synaptic depression (LTD) in cortico-striatal circuits is initiated by depolarization of striatal medium spiny neurons through a convergent cortical glutamatergic input. This produces retrograde endocannabinoid signaling to presynaptic cortical terminals and eventually results in long term (>30 minutes) decreases in glutamate release. These same circuits can also undergo short-term depression (STD) through a less well-defined process in which the magnitude of postsynaptic responses returns to baseline levels within 10 minutes. Additionally, the cortico-striatal circuit shows characteristics of a GABA_A receptor-dependent low-pass filter, which results in significant attenuation of high frequency cortical inputs. The majority of *in vitro* studies of LTD have used a 100-Hz induction paradigm and it is unclear whether other frequencies, which may also have physiological relevance, have equivalent ability to induce this form of plasticity. Here we have investigated the effectiveness of a range of induction paradigms in producing LTD in cortico-striatal circuits, and demonstrate that some lower frequency paradigms, with perhaps more physiological relevance, are more effective at inducing LTD. We also show that GABA_A receptor-dependent frequency filtering in this circuit is altered following the induction of LTD and STD suggesting an important role for synaptic depression in signal processing in these circuits.

Key Words

Frequency filtering

Induction paradigm

Short-term depression (STD)

Long-term depression (LTD)

1. Introduction

Synaptic plasticity underlies nervous system functions from signal processing to learning and memory (Neves et al., 2008; Song et al., 2000). Short-term changes occurring in presynaptic neurons and in feedback circuits underlie the frequency dependency and immediate efficacy of transmission of action potentials through neuronal networks, while long-term changes in both the pre- and post-synaptic regions are thought to be the basis of learning and memory (Kreitzer, 2009; Lovinger, 2010). The glutamatergic synapse between layer V cortical projection neurons and medium spiny neurons (MSNs) in the dorsal striatum exhibits both of these forms of plasticity. Importantly, cortico-striatal circuits play an important functional role in various forms of associative learning and in modulation of motor behavior (Balleine et al., 2007).

The induction of long-term synaptic depression in cortico-striatal circuits is initiated by convergent glutamatergic (layer V) and dopaminergic (substantia nigra) inputs to MSNs leading to their depolarization and the activation of post-synaptic metabotropic glutamate receptors (mGluRs) and dopamine type-2 receptors (D2Rs). Simultaneous depolarization of MSNs and activation of these receptors leads to the production of endocannabinoids by MSNs, which retrogradely bind pre-synaptic CB1 receptors on glutamatergic terminals leading to long-term decreases in the probability of glutamate release – this phenomenon is characterized as long-term depression (LTD) (Surmeier et al., 2007). Descriptions of this form of LTD in cortico-striatal circuits have been predominantly based upon a high frequency induction paradigm using 3 to 4 trains of 100-Hz stimuli at maximum stimulus intensity (Calabresi et al., 1992). We have previously investigated the role of D2 and CB1 receptors in the induction of LTD following this 100-Hz high frequency stimulus paradigm (Baca et al., 2013) and have confirmed the occurrence of striatal LTD, and the role of both receptors in the LTD induction process.

This 100-Hz high frequency induction paradigm, through repetitive firing of glutamatergic fibers with coincident dopaminergic input, produces an initial post tetanic depression (PTD) followed in some instances by a process referred to here as short-term depression (STD), which is characterized by a return within about 10 minutes to response amplitudes equivalent to baseline levels prior to the induction paradigm. In other instances, a longer lasting LTD is induced, which is maintained for an excess of 30 minutes (Lovinger et al., 1993) and is characterized by significantly attenuated response amplitudes (Baca et al., 2013). The dependence of STD and LTD upon the frequency and magnitude of the induction paradigm

has not been extensively investigated. One of the few published studies that examined the influence of the stimulus frequency on the induction of LTD in the striatum used a 10-Hz, 5-minute stimulus train (Ronesi and Lovinger, 2005) instead of the 100-Hz induction paradigm commonly employed. This form of LTD, unlike that induced with the 100-Hz paradigm, does not appear to depend on post-synaptic depolarization or activation of mGluRs.

In vivo studies have shown that long term potentiation (LTP) is induced in striatal circuits with sustained focal 5-Hz stimulation of 500-1000 pulses (Charpier et al., 1999), but similar *in vivo* conditions for LTD induction have not been clearly established. It is known that layer V cortical inputs to striatal MSNs contain regular spiking (RS) and intrinsic bursting (IB) cells. *In vivo* measurements have shown that the mean frequency of spikes for IB cell burst firing is ~77 Hz and it is less for RS cells (Degenetais et al., 2002), so inputs from IB cells may provide high frequency input to striatal MSNs. Intra-somatic current injection of between 100 – 400 pA into patch-clamped dopamine D1- or D2 receptor-expressing MSNs reveals firing rates that are less than about 15 Hz for D1 MSNs and less than about 25 Hz for D2 MSNs, indicating that D2 MSNs are more excitable than D1 MSNs (Gertler et al., 2008). Recently, *in vivo* recordings of striatal MSNs showed baseline firing rates of ~4.5 Hz for these neurons (Jin et al., 2014). During a task in which mice learned rapid action sequences by pressing a lever, it was found that MSNs fell into two populations one that demonstrated increases in baseline firing rates ranging from ~10 - 20 and another that showed decreases in firing rates below ~2 Hz (Jin et al., 2014). While the relationship of these rapid action sequence tasks to striatal plasticity has not been established, it does suggest the probable range of firing frequencies of MSNs during baseline and task-specific conditions. To better understand the role of long-term synaptic plasticity in cortical inputs to striatal MSNs, it is important to characterize the effectiveness of physiologically-relevant frequencies in the induction of STD and LTD. We therefore explored the relationship of the magnitude or occurrence of LTD and STD to the frequency and magnitude of the input stimulus when delivered at frequencies between 10 Hz and 100 Hz.

Following such an induction paradigm, the resulting LTD or STD in MSNs would be expected to affect subsequent transmission of information through the cortico-striatal circuit. We have previously demonstrated low-pass filter characteristics of layer V glutamatergic inputs to MSNs in the striatum (Jelinek and Partridge, 2012) and provided evidence that GABAergic feedback mechanisms underlie these characteristics. We have also previously shown differential

filtering characteristics within the CA3, CA1, and dentate gyrus of the hippocampus (Scullin and Partridge, 2012), indicating that filter properties are a local property of specific brain regions. If this low-pass filtering and synaptic plasticity are a characteristic feature of the cortico-striatal circuitry, it is an interesting and important question to examine how these phenomena may interact. In this study, we have extended our previous findings to investigate the effect of the induction of LTD or STD in cortico-striatal synapses on the inherent low-pass filter characteristics of the striatal circuit.

2. Results

2.1 Comparison of Frequency Paradigms and LTD Induction

Extracellular field excitatory postsynaptic potential (fEPSP) population spike (PS) amplitudes were measured in the medial portion of the dorsal striatum in coronal slices of P21 – P28 C57/Bl6 mice. To characterize the role of the frequency and magnitude of the input stimulus on the induction of synaptic depression, six distinct induction paradigms, which are outlined in Table 1, were used (see Experimental Procedures for details).

The low frequency continuous stimuli induction paradigms (#1 and #2) were included in order to make comparisons with previous data (Lerner and Kreitzer 2012; Kreitzer and Malenka, 2005; Ronesi and Lovinger, 2005), which suggest that there may be different mechanisms involved in the induction of STD or LTD at this frequency. The 400 stimuli induction paradigms (#3, #4, and #5) were included in order to assess the influence of the frequency with a fixed number of total stimulus pulses. Both half maximum and maximum stimulus intensities (stimulus current inputs to obtain half maximum or maximum PS outputs) were used in several of the induction paradigms in order to determine the influence of the magnitude of the stimulus on the induction of STD or LTD.

Following all of the induction paradigms examined, PS amplitudes exhibited an initial depression to ~20-30% of baseline (post-tetanic depression, PTD). For some slices, the PS amplitude returned to near baseline levels within a few minutes, while in other slices it remained depressed for the full 30-minute recording period (e.g. Fig. 1A). We used a cluster analysis (see Experimental Procedures) to differentiate between two distinct populations, which were operationally defined as STD and LTD based on the percent of PS amplitude recovery after 30 minutes in comparison to baseline PS amplitudes measured for 10 minutes prior to the induction

paradigm. Fig. 1B shows the aggregate data from the experiments prior to cluster analysis as well as the clustered data of the percent PS amplitude recovery following the induction paradigm #3 for the STD and LTD populations. Fig. 1C is a plot of representative PS amplitudes for the 10-minute baseline period and for the 30-minute recovery period following the induction paradigm for slices that were assigned to the STD and LTD populations. Each of the remaining induction paradigms generated plasticity that could also be differentiated into distinct STD and LTD populations similar to those shown in Fig. 1B.

We also performed a set of control experiments in which an induction paradigm was not delivered, in order to assess the stability of PS amplitudes during a 40-minute recording period. In these control experiments, the PS amplitudes at the end of the 40-minute period were found to remain >95% of the baseline levels and cluster analysis revealed a single population similar to that defined as STD. The magnitude of PS amplitudes of control slices were used for comparison with changes in PS recovery by each of the induction paradigms used in these experiments.

We next assessed separately whether the presence of these two forms of synaptic plasticity (LTD and STD) differed among the various induction paradigms examined. A multiple comparisons ANOVA ($F_{6,53}=28.53$, $p<0.0001$) of the LTD populations showed significance among the different induction paradigms examined and controls without a preceding induction (Fig. 2A). As expected, post-hoc analysis of all of the LTD populations showed significantly less recovery 30 minutes after the induction paradigm when compared to controls (Fig. 2A). Additionally, post-hoc analysis showed that the continuous maximum intensity induction paradigm #1 generated more robust LTD populations with significantly less recovery after 30 minutes than was generated by the other induction paradigms (data not shown). A multiple comparisons ANOVA ($F_{6,60}=3.09$, $p<0.01$) did not show significant differences among the different induction paradigms for STD populations and controls without a preceding induction (Fig. 2B) indicating that all STD populations recover to an amplitude that is equivalent to controls in which synaptic plasticity was not induced. We next assessed the occurrence of STD and LTD following the 10-Hz, 30-Hz, and 100-Hz maximum input paradigms #3, #4, and #5, since each of these paradigms included the same number of stimuli and was delivered at the same maximum input. The 10-Hz paradigm #3 preferentially induced LTD over STD in 56% (10/18) of slices, versus 41% (14/34) for the 30-Hz paradigm #4, and 31% (5/16) for the 100-Hz

paradigm #5. For comparison purposes, paradigm #1 preferentially induced LTD over STD in 76% (13/17) of slices, versus 31% (4/13) for paradigm #2 and 47% (7/15) for paradigm #6. However, comparison of the frequency of LTD induction between pairs of paradigms did not reveal significance (#3 versus #4; $p=0.14$; #4 versus #5; $p=0.20$; #3 versus #5; $p=0.10$; all Fisher's exact test).

2.2 LTD and STD alter frequency filtering in 10-Hz LTD paradigm

In a recent study, we assessed the frequency response of cortico-striatal circuits and demonstrated that low-pass filter characteristics are dependent on GABAergic feedback, since use of a GABA_A receptor antagonist minimizes the roll-off at frequencies above 2 Hz (Jelinek and Partridge, 2012). Since the STD and LTD clusters appear to represent divergent forms of plasticity among slices, it was important to test whether there were differences in the low-pass filtering characteristics of the STD and LTD slice populations following the induction of plasticity. We chose the 10-Hz 400 stimuli maximum stimulus paradigm (#3) to assess the effect of plasticity on the low-pass filter characteristics of this synapse, since this induction paradigm was the most effective in inducing LTD.

Fig. 3A shows the time course of PS amplitudes for the STD and LTD slice populations following induction by the 10-Hz 400 maximum stimulus paradigm (#3). PS amplitudes were measured at the following times: BL#1 - an initial 10-minute baseline recording at half maximum intensity; RS#1 - the first assessment of the frequency response; BL#2 - a second 10-minute baseline recording at half maximum intensity; IND - induction paradigm (#3); post-IND response - a 30-minute recording at half maximum intensity; and finally RS#2 - a second assessment of the frequency response. As shown in Fig. 3C, the ratio of the PS amplitudes during BL#2 to that during BL#1 was close to unity for both the STD and LTD populations, indicating that there was not a significant synaptic depression induced during the first assessment of the frequency response. As shown in Fig. 3D, cluster analysis revealed distinct STD and LTD populations during the post-induction response period before the second assessment of the frequency response (RS#2). Although PS amplitudes were greatly reduced in the LTD population, they were of sufficient amplitude for frequency response assessment.

To determine the effect of the type of synaptic depression on the low-pass filter properties, the responses to RS#1 were compared with the responses to RS#2. Since RS#1

occurred prior to the induction paradigm, analysis of RS#1 included both slices that received a subsequent induction paradigm regardless of whether it produced LTD or STD. Analysis of RS#2 was performed separately on the STD and LTD populations. Fig. 3B shows a Bode magnitude plot of the normalized amplitudes of PS responses to frequencies between 2 and 60 Hz. As shown in Table 2, a least squares regression fit of RS#1 data to a 3rd order Butterworth low-pass filter (see Experimental Procedures equations 1 & 2) yielded a corner frequency (CoF, equation 2) of 4.37 Hz and a stop band slope parameter (A, equation 1) of 0.0185. A similar fit of the RS#2 STD cluster yielded an increased CoF and a larger A (Table 2), indicating a prominent increase in the cut off frequency and slight shift in the stop band slope compared to RS#1. Table 2 indicates that a least squares regression fit to a 3rd order Butterworth low-pass filter for the RS#2 LTD cluster yielded a similar value for the CoF and a smaller A. To better quantify these differences in filter characteristics, we compared the frequency-dependent PS amplitudes for the RS#1 and RS#2 STD and LTD clusters at the 20.8 Hz, 35.2 Hz, and 57.5 Hz points on the Bode magnitude plot. As shown in Fig. 3E, at 20.8 Hz, no significant difference was found between RS#1 and RS#2 STD and LTD clusters. At 35.2 Hz, the RS#2 PS amplitudes of the STD and LTD clusters were both significantly greater than those of the RS#1 cluster. At 57.2 Hz, the RS#2 PS amplitude of the LTD cluster was significantly greater than that of either the RS#2 or the RS#1 STD cluster.

A set of control experiments was carried out using the same experimental paradigm as shown in Fig. 3A, but without imposition of the induction paradigm after BL#2. We found no significant differences in the magnitude of PS amplitudes representing the BL#1, BL#2, or post-induction response groups after 30 minutes, (data not shown), indicating that there was no run down of PS amplitudes over this time period. As shown in Table 2, a least squares regression fit of these data to a 3rd order Butterworth low-pass filter gave a similar value of the CoF and slope band parameter A for both RS#1 and RS#2 controls. These values were comparable to those found for RS#1 in those experiments in which an induction paradigm was applied.

2.3 Frequency filtering removed in 10-Hz LTD paradigm by GABA_A receptor antagonist

We next repeated the 10-Hz 400 maximum stimuli (#3) experiments in the presence of 20 μ M picrotoxin, a GABA_A receptor antagonist. As before, cluster analysis identified distinct STD and LTD populations at the 30-minute time period following the induction paradigm. However,

there was not a significant least squares fit of the data to a 3rd order Butterworth low-pass filter, in either the RS#1, RS#2 STD, or RS#2 LTD populations as shown in Table 2. This indicates a major GABA_A receptor-dependent component of the low-pass filter characteristic (Fig. 4A) as we had previously observed (Jelinek and Partridge, 2012), both during RS#1, but also following the induction paradigm (Fig. 4A). As shown in Fig. 4B, the ratio of the PS amplitudes during BL#2 to that during BL#1 was close to unity for both the STD and LTD populations, indicating that there was not a significant synaptic depression induced by the first random stimulus paradigm. As shown in Fig. 4C, cluster analysis revealed distinct STD and LTD populations during the post-induction response period in the presence of GABA_A receptor blockade. In the presence of a GABA_A receptor antagonist, the magnitude of the STD cluster (0.8391 +/- 0.0481; Fig. 4C) decreased in comparison to that of the STD cluster in the previous experiments without a GABA_A receptor antagonist (0.9271 +/- 0.0638; Fig. 3D) although the difference was not significant (one way ANOVA $F_{1,10}=0.347$, $p=0.57$). In the presence of a GABA_A receptor antagonist, the magnitude of the LTD cluster (0.4755 +/- 0.0467; Fig. 4C) decreased in comparison to that of the LTD cluster in the previous experiments without a GABA_A receptor antagonist (0.6009 +/- 0.0297; Fig. 3D) again the difference was not significant (one way ANOVA $F_{1,18}=0.951$, $p=0.18$). The 10-Hz paradigm without a GABA_A receptor antagonist preferentially induced LTD over STD in 68% (15/22) of slices and, in the presence of a GABA_A receptor antagonist, LTD was induced over STD in 50%, (5/10) of slices. However, a comparison of the frequency of LTD induction did not reveal significance ($p=0.19$; Fisher's exact test).

3. Discussion

Basic cellular mechanisms implicated in both long term and short-term plasticity have been extensively investigated in cortico-striatal systems. To be a valid model, this plasticity must be initiated by a physiologically-relevant neural signal consistent with observed *in vivo* firing patterns of cortical inputs and short and long term changes in output striatal MSNs. To date, a 100-Hz high frequency induction paradigm has been used predominantly to characterize the transmitter systems and receptors, which underlie cortico-striatal long term plasticity including LTD, STD, and LTP (Kreitzer, 2009; Lovinger, 2010). This stimulus frequency is equivalent to the upper limit of the gamma frequency band (30 - 100 Hz); however, EEG measurements identify the beta frequency band (13 - 30 Hz) as being the most common

frequency range of an alert awake brain (Ioannides et al., 2004; Stern and Engel, 2013). Slice recordings of striatal MSNs indicate that they remain in the down state with a very low (0.1 – 1 Hz) intrinsic firing rates unless an input stimulus causes a transition to an up state with short-term burst firing in the 15-25 Hz range (Gertler et al., 2008). Thus striatal MSNs do not typically fire in the range associated with the experimental induction of LTD or STD and since cortico-striatal plasticity has not been extensively studied *in vivo*, behaviorally relevant frequencies of cortical inputs to striatal MSNs still remain to be established. Of special importance is the effect of natural inputs on cortico-striatal plasticity and the role that this plasticity serves in striatal-specific behaviors.

As described in the introduction, striatal MSNs receive convergent glutamatergic inputs that depolarize them and activate mGluRs as well as dopaminergic inputs that activate D2Rs. These convergent inputs are necessary to produce endocannabinoids that act pre-synaptically on CB1Rs, in order to initiate the induction of LTD. Lack of either sufficient glutamatergic or dopaminergic input may not allow sufficient production of endocannabinoids for the induction of LTD, but might still produce STD. In a given brain slice recording, the fEPSP depends on the location of the stimulating and recording electrodes and hence the population of MSNs that generate the underlying current sources and sinks. Local differences in the effectiveness of glutamatergic and dopaminergic inputs to MSNs may thus determine the type of plasticity observed.

In this study we have compared the induction of LTD and STD by paradigms with frequencies ranging from 10 Hz to 100 Hz. We found that the 10-Hz 400 stimuli paradigm (#3) favored LTD over STD more than the same number of stimuli at higher frequencies (paradigms #4 and #5) (Fig. 2), although the resulting magnitudes of LTD and STD were similar. The 10-Hz 3000 stimuli paradigm #1 produced a more robust level of LTD than paradigms #3, #4, or #5 with 400 stimuli (Fig. 2A).

Two of our induction paradigms compared the use of ½ maximum stimulus intensity to maximum stimulus intensity. While no significant differences were found between ½ maximum (#6) and maximum (#5) stimulus intensity for 400 stimuli at 100 Hz, a more robust LTD was found for maximum (paradigm #1) than ½ maximum stimulus intensity (paradigm #2) for 3000 stimuli at 10-Hz (Fig 2). Importantly, the stimulus intensity is less important in the 100-Hz paradigms, which have been used predominately in previous studies of LTD, than in lower

frequency induction paradigms. Our findings that a 10-Hz induction paradigm produces more robust forms of LTD may be relevant given the recent findings that *in vivo* firing rates ranged from ~10 - 20 Hz in some MSNs (Jin et al., 2014), indicating that 10-Hz may be a physiologically relevant firing frequency for MSNs to undergo LTD. Differences in the ability to undergo LTD in cortico-striatal circuits have been shown to be associated with the acquisition and consolidation phases of a skilled learning task (Yin et al., 2009). In that study, *in vivo* multi-electrode array recordings of the dorsal medial striatum (DMS) and dorsal lateral striatum (DLS) MSNs were made in mice while they learned a rotorod task during repeated trials for several days. Differences were found in the ability to undergo LTD in both the DLS and DMS MSNs depending on the phase of the learning task. Another recent study investigated electrophysiological responses in knockout mice deficient in adenylyl cyclase 5 (AC5) during behavioral tasks. This subtype of adenylyl cyclase is highly expressed in MSNs, where it serves as a downstream effector of D2Rs, which play a role in LTD (Kheirbek et al., 2009). The AC5 knockout mice failed to undergo LTD in association with a learned task, further demonstrating how behavioral changes may be correlated with changes in LTD.

In the study reported here, we extended our previous findings of a low-pass filter characteristic for layer V cortical inputs to MSNs (Jelinek and Partridge, 2012; Table 2) to intervals before and after the 10-Hz 400 maximum input induction paradigm (#3, Fig. 3B; grey) and further showed that low-frequency filtering in both STD and LTD populations depends on feedforward GABAergic interneurons with input from cortical afferents and direct GABAergic feedback through branches to adjacent MSNs. While both of these pathways presumably contribute to low frequency filtering it has been shown that the feedforward pathway is the most common and effective (Tepper et al., 2008; Table 2). The STD population showed a distinct shift in the low-pass filter as characterized by an increase in the CoF from 4.4 Hz to 14.2 Hz (Table 2). In contrast, the CoF of the LTD population did not change significantly from its baseline value of 3.44 Hz, but this population displayed a reduction in its stop band roll off parameter A (Fig. 3B; green). These results demonstrate that the differential exhibition of LTD vs. STD in slices is correlated with different initial low-pass filter characteristics. Since the low-frequency filter characteristics are expected to play an important role in sculpting the transmission of signals through the cortico-striatal pathway, these observations suggest an important role that the conditions of synaptic plasticity may play in the frequency dependence of signal transmission.

An interesting study would be to determine whether this reflects an intrinsic difference in the particular cortico-striatal synapses that is independent of subsequent induction of long-term plasticity or if it directly relates to the induction of either LTD or STD. Further research is necessary to distinguish between these possibilities.

We found a decrease in the proportion of slices in which LTD was induced in the presence of picrotoxin, which is consistent with the finding that GABAergic synapses onto MSNs are more likely to undergo LTD than glutamatergic synapses onto MSNs (Adermark and Lovinger, 2009). As a result GABAergic MSNs may provide feedback inhibition by increasing the likelihood that they undergo LTD when they receive inputs from other GABAergic MSNs.

We have shown here that the likelihood of inducing LTD or STD in cortico-striatal circuits is dependent on the characteristics of the induction paradigm. Additionally we have shown that the low-pass filter characteristics of cortico-striatal circuits undergo alteration as a result of LTD and STD in a GABAergic feedback-dependent manner. Future studies of how *in vivo* frequency alterations of MSNs coincide with striatal plasticity are needed to confirm whether cortico-striatal LTD and STD occur differentially in response to behaviorally-relevant firing.

4. Experimental Procedure

Animals

All experiments were approved by the Institutional Animal Care and Use Committee at the University of New Mexico Health Sciences Center Laboratory Animal Care and Use Committees and the National Institutes of Health. Electrophysiology experiments were performed on coronal striatal slices prepared from 21 - 28 day old male mice using standard techniques (Schiess et al., 2006).

Electrophysiological Measurements

Briefly, animals were deeply anaesthetized by I.P. injection of 250 mg kg⁻¹ ketamine, brains were rapidly removed, and coronal cortico-striatal slices cut at 300 μ m with a vibroslicer (Pelco 101, St Louis, MO, USA) in an ice bath with a cutting solution containing (mM): 220 sucrose, 3 KCl, 1.2 NaH₂PO₄, 26 NaHCO₃, 12 MgSO₄, 0.2 CaCl₂, 10 glucose and 0.01 mg ml⁻¹ ketamine equilibrated with 95%O₂–5%CO₂. Slices were then transferred to artificial

cerebrospinal fluid (ACSF) containing (mM): 126 NaCl, 3 KCl, 1.25 NaH₂PO₄, 1 MgSO₄, 26 NaHCO₃, 2 CaCl₂ and 10 glucose equilibrated with 95% O₂–5% CO₂ at 30°C for 1 h and then maintained at room temperature until recording in a constant flow chamber (Warner Instruments, Hamden, CT, USA or Scientific Systems Design, Mercerville, NJ, USA) maintained at 32°C and continuously perfused at 2 ml min⁻¹ with ACSF saturated with 95% O₂–5% CO₂.

Standard electrophysiological techniques were used for field potential (fEPSP) population spike (PS) recordings (Schiess et al., 2006) following stimulation of cortical layer V afferents at the dorsal border of the striatum. To provide consistency within our studies and with recent studies that compared dorsolateral and dorsomedial striatum (Yin et al., 2009) we restricted our recordings to the dorsomedial striatum approximately 0.5 - 1.0 mm from the corpus callosum border. Importantly, other studies of striatal plasticity have not distinguished between these two regions (e.g. Calabresi et al., 1992). The resulting PSs represent predominately activity of medium spiny neurons (MSNs) (Surmeier et al., 1996). Presynaptic constant current pulses (150 μ s duration) were applied at 20 second intervals with an Iso-Flex constant current stimulator (API Instruments, Jerusalem, Israel) through a concentric bipolar electrode (FHC, Bowdoinham, ME, USA) at a stimulus intensity (0.1 – 2.0 mA) adjusted to produce 50% of the maximum population spike amplitude, determined by an input-output series prior to the start of each experiment. PSs were recorded with an Axoclamp 2B (Molecular Devices, Sunnyvale, CA), amplifier and a Digidata 1322A interface using pCLAMP 9.2 software (Molecular Devices) for experimental control and data analysis. Recordings were digitized at 500 kHz and filtered at 2 kHz. PS amplitudes were calculated by subtracting the average of both positive components in the fEPSP from the maximum intervening negative value.

Plasticity Induction Paradigms

To assess long-term synaptic plasticity in the cortico-striatal field, a 10-minute baseline was established at ½ maximum stimulus intensity, then one of six LTD induction paradigms (Table 1) with stimulus pulses at either half maximum or maximum intensity was applied, and finally 30 minutes of recordings were obtained again at ½ maximum stimulus intensity. The average amplitude of the final 10 PSs during the 30-minute period was compared to the average amplitude of 31 PSs during the 10-minute pre-HFS baseline in order to determine the percentage of change relative to the baseline. Cluster analysis (described below) was used to differentiate

STD and LTD populations resulting from each induction paradigm. The six LTD induction paradigms (Table 1) consisted of the following: 4 sets of 10-, 30-, or 100-Hz pulse trains (each train 100 pulses each) at maximum stimulus intensity (except 100 Hz where experiments were performed at half maximum intensity as well) separated by 1 minute for 400 stimuli total; or a continual 5-minute train of 10 Hz pulses at half maximum or maximum stimulus intensity for 3000 stimuli total.

Frequency Response Assessment

The filter properties of the cortico-striatal field were determined by an additional stimulus protocol, which determined PS amplitudes as a function of the instantaneous stimulus frequency. Stimulus frequencies were presented in a random order to minimize effects resulting from sequentially increasing or decreasing frequencies. In this random frequency train protocol, nine sets of 5- to 60-Hz instantaneous frequency pulse trains with the order of the frequencies randomly distributed in each set were produced by a waveform stimulus file in Clampex (Molecular Devices, Sunnyvale, CA).

Frequency response data for PS amplitudes were fit with a 3rd order Butterworth low-pass filter with a least squares regression using the Levenberg-Marquart method in ProStat (v 6.5, Poly Software International, Pearl River NY) using equations 1 and 2. The Butterworth filter was chosen because its flat passband response best represents our measured frequency response data.

$$V_{mean} = A \times \log_{10}(1 + fr^6) \dots (1)$$

$$fr = \frac{Hz}{CoF} \dots (2)$$

Where: V_{mean} is the frequency-dependent gain that was fit to the average PS amplitude during the binned PS amplitudes generated by the random frequency protocol, Hz is the stimulus frequency, CoF is the cutoff frequency for a low-pass filter, and A is a scaling factor that is directly proportional to the roll off of the filter.

To assess changes in frequency filtering characteristics during long-term synaptic plasticity in the cortico-striatal field, the 10-Hz 400 stimuli maximum stimulus intensity

paradigm was used in the following sequence: (a) an initial 10-minute baseline was established at $\frac{1}{2}$ maximum stimulus intensity, (b) the first assessment of the frequency response described above was delivered, (c) a second 10-minute baseline was established at $\frac{1}{2}$ maximum stimulus intensity, (d) the 10-Hz stimulus (IND) paradigm above (400 pulses) with stimulus pulses at $\frac{1}{2}$ maximum or maximum intensity was applied, (e) 30 minutes of post-induction recordings were obtained again at $\frac{1}{2}$ maximum stimulus intensity and (f) the second assessment of the frequency response described above was delivered. The average amplitude of the final 10 PSs during the 30-minute period (f) was compared to the average amplitude of 31 PSs during the initial 10-minute pre-IND baseline (a) in order to determine the percentage of change relative to the baseline, and cluster analysis as described below was used to further differentiate distinct STD and LTD populations from these results. A comparison of the average amplitude of 31 PSs during the second 10-minute pre-IND baseline (c) to the first 10-minute pre-IND baseline (a) was also made as a control to test if the assessment of the frequency response introduces changes in the PS amplitudes prior to the plasticity induction paradigms.

Drugs

Picrotoxin (Tocris, Ellisville, MO, USA) was stored frozen in aliquots and diluted to the appropriate concentration in ACSF for electrophysiology on the day of the experiment.

Statistical Analysis

SPSS 16.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis of data. Numerical values for data measurements are expressed as the mean $\pm$ standard error unless otherwise specified. Statistical p values were represented as follows: * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$. Significance determined by an ANOVA analysis was followed by a Scheffe's post-hoc test to determine the p values between the groups compared in the experiments. Comparisons of groups were made with an ANOVA and all post-hoc tests were Scheffe's unless otherwise noted.

A two-step cluster analysis with no pre-determined number of clusters (SPSS 16.0) was used for the various induction paradigm experiments to group the results into STD and LTD population clusters. The two-step cluster analysis utilizes a Student's t -test with a Bonferroni adjustment applied to statistically discriminate clusters. Cluster analysis identifies clusters within a data set that minimize within-group variation and maximize between-group variation.

The result of the cluster analysis is the identification of either a single cluster or several (2 or more) clusters within the data set as well as the mean and standard deviation for each identified cluster.

Author Contributions:

L.D.P, M.B., A.R.S. and C.J., conceived of the project. M.B, A.R.S., and D.J. performed the electrophysiology experiments. A.R.S and C.J. wrote Matlab software to analyze the data. L.D.P. supervised and helped in the interpretation of the electrophysiology. M.B and L.D.P wrote and M.B, L.D.P. and C.J. edited the manuscript.

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Figures & Table Legends

Fig. 1: Cluster analysis determination of 10-Hz 400 stimuli maximum input induction paradigm (#3).

A Representative example of population spikes traces before (gray) and after (colored) in 10-Hz 4×1 s maximum input induction paradigm (#3) for STD population ($> 70\%$ recovery, $n = 8$, red) and LTD population ($< 60\%$ recovery, $n = 10$, blue) as determined by cluster analysis. **B** Mean $\pm$ SEM of the 30-minute post-induction response pop spike (PS) amplitude as a percent of the baseline PS amplitude for STD and LTD populations compared to the aggregated non-clustered results. **C** Time course of normalized population spike amplitudes for LTD and STD populations as determined by cluster analysis, showing timeframe for recordings for baseline (10 minutes), induction paradigm, and post-induction responses (30 minutes).

Fig. 2: Comparison of LTD and STD clusters in different induction paradigms.

A Population clusters with least post-stimulus response recovery (LTD - blue). Percent post-stimulus response of population spike amplitude for clusters with the least recovery for each of the 6 induction paradigms (Table 1) compared to controls (no induction). Solid colors represent maximum input stimulus and hatched colors represent half maximum input stimulus used during frequency induction. **B** Population clusters with greatest post-stimulus response recovery (STD - red). Percent post-stimulus response of population spike amplitude for clusters with the greatest recovery for each of the 6 induction protocols (Table 1) compared to controls (no induction). Solid colors represent maximum input stimulus and hatched colors represent half maximum stimulus used during frequency induction paradigms. Statistics: ANOVA multiple comparisons and Scheffe's post hoc tests. (** $p < 0.01$; *** $p < 0.001$)

Fig. 3: Effect of long-term plasticity on cortico-striatal frequency responses to 10-Hz 400 stimuli maximum input induction paradigm (#3).

A Time course of normalized population post-induction response spike amplitudes separated into 2 groups determined by cluster analysis: STD (red) and LTD (blue). A 10-minute baseline (BL#1) was followed by a random stimulus paradigm (RS# 1), followed by a second 10-minute baseline (BL#2). Next a 10-Hz, 400 stimuli induction paradigm (#3) was performed, followed by a 30-minute post-induction response (Post-IND Response). Finally, this was followed by an identical random stimulus paradigm (RS#2). Averages of the last 10 readings 30 minutes after the induction stimuli were divided by averages

of the initial baseline (BL#1) to obtain the % of recovery of the pop spike following the induction stimulus. Cluster analysis of the ratio of post-IND response/BL#1 of each result was used to obtain STD and LTD populations. **B** Frequency filtering properties for 10-Hz, 400 stimuli at maximum intensity. A 3rd order Butterworth low-pass filter fits the RS#1 and RS#2 paradigms. Clusters: RS#1 (grey), corner frequency (CoF) = 4.3688 Hz, A=0.0185, n=22; RS#2 STD cluster (red): CoF = 14.2026 Hz, A = 0.0370; n = 7; RS#2 LTD cluster (blue): CoF = 3.4474, A=0.0084; n=15. **C** Ratio of 10-minute BL#2 to BL#1 for STD and LTD clusters; STD (mean 0.8978 +/- 0.0239, red) and LTD (0.9939 +/- 0.0688, blue). **D** Ratio of STD (mean 0.9271 +/- 0.0638, red) and LTD (0.6009 +/- 0.0297, blue) clusters **E** Comparison of RS#1 (grey), RS#2 STD cluster (red), and RS#2 LTD cluster (blue) frequency responses at 20.8 Hz, 35.2 Hz and 57.5 Hz. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

Fig. 4: Effect of long-term plasticity on cortico-striatal frequency responses to 10-Hz 400 stimuli maximum input induction paradigm (#3) in the presence of GABA_A receptor antagonist. **A** Frequency responses for 10-Hz, 400 stimuli at maximum intensity. Responses not found to fit 3rd order Butterworth low-pass filter for random stimulus RS#1 and RS#2 paradigms. Clusters: RS#1 (grey); n=10, RS#2 STD cluster (red); n=5, RS#2 LTD cluster (blue); n=5. **B** Ratio of 10-minute baseline responses BL#2 to BL#1 for STD and LTD clusters STD (mean 0.8965 +/- 0.0474, red) and LTD (0.7744 +/- 0.0260, blue). **C** Ratio of STD cluster (mean 0.8391 +/- 0.0481, red) and LTD cluster (0.4755 +/- 0.0467, green) of 30-minute post-induction responses to initial 10-minute baseline responses (BL#1). (** $p < 0.01$)

Table 1: Six LTD paradigms used to compare LTD and STD populations. Stimulus frequency, pulse trains, total number of pulses, input stimulus current, and the number of slices which produced STD and LTD respectively for each paradigm.

Table 2: Corner Frequency (CoF) and stop band parameter (A) determination for random stimulus experiments. CoF, A parameters, and numbers of slices for 10 Hz LTD paradigm experiments for random stimulus paradigms #1(RS#1) and #2 (RS#2), controls lacking 10 Hz frequency trains, and in the presence of 20 μ M picrotoxin. (ND = not determined)

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Figure 1

Figure 2

Figure 3

Figure 4

LTD Paradigm	Frequency (Hz)	Trains	# of Pulses	I_{stim}	STD #	LTD #
#1	10	5 min continuous	3000	max	4	13
#2	10	5 min continuous	3000	half max	9	4
#3	10	4 ×100 pulses, 1 min delay	400	max	8	10
#4	30	4 ×100 pulses, 1 min delay	400	max	20	14
#5	100	4 ×100 pulses, 1 min delay	400	max	11	5
#6	100	4 ×100 pulses, 1 min delay	400	half max	8	7

Table 1: Six LTD paradigms used to compare LTD and STD populations. Stimulus frequency, pulse trains, total number of pulses, input stimulus current, and the number of slices which produced STD and LTD respectively for each paradigm.

Experiment	CoF (Hz)	A	#
10 Hz RS#1	4.37	0.0185	22
10 Hz RS#2 STD	14.20	0.0370	7
10 Hz RS#2 LTD	3.45	0.0084	15
10 Hz RS#1 Control	5.15	0.0190	7
10 Hz RS#2 Control	5.68	0.0170	7
10 Hz RS#1 picrotoxin	ND	ND	14
10 Hz RS#2 STD picrotoxin	ND	ND	5
10 Hz RS#2 LTD picrotoxin	ND	ND	5

Table 2: Corner Frequency (CoF) and stop band parameter (A) determination for random stimulus experiments. CoF, A parameters, and numbers of slices for 10 Hz LTD paradigm experiments for random stimulus paradigms #1(RS#1) and #2 (RS#2), controls lacking 10 Hz frequency trains, and in the presence of 20 μ M picrotoxin. (ND = not determined)