THETA-BURST LTP IN THE DORSAL STRIATUM: ITS DEVELOPMENT AND USE TO ILLUMINATE STRIATAL DYNAMICS UNDERLYING SKILL LEARNING, AND THE ABERRANT LEARNING BEHIND ADDICTION.

by

Sarah Louise Hawes

A Dissertation
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Theta-burst LTP in the Dorsal Striatum: Its Development and Use to Illuminate Striatal Dynamics Underlying Skill Learning, and the Aberrant Learning behind Addiction.

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at George Mason University

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DEDICATION

I dedicate this dissertation to my grandfather Cecil Davis Johnson, who loves good research and bad jokes.

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I would like to thank my committee for their guidance, training and support. Dr. Dan Cox has been consistently helpful and generous with equipment and also with scientific and career advice. This remained true even while his own career sky-rocketed him through three labs and two universities in the time my dissertation transpired. I wanted Dr. Ted Dumas on my committee after having him as a course instructor and recognizing his rigorous expectations for considering and presenting research. He has always been a great resource for practical advice from a hands-on experimentalist, and I learned a lot from interacting with his lab. I learned striatal field recording in the lab of Dr. David Lovinger, who has been an expert advisor on experimental design and presentation. His creativity, veracity and relaxed nature make him an ideal investigator and a strong ally in the unpredictable field of striatal plasticity.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATIONS

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ..... AMPA
Acetylcholine(rgic) ..... ACh
Action potential ..... AP
AMPA receptor ..... AMPAR
Artificial cerebrospinal fluid ..... aCSF
A-type potassium channel ..... KA
cAMP activated protein kinase ..... PKA
Cre recombinase ..... Cre
current-frequency ..... IF
Input resistance ..... IR
current-voltage IV
cyclic adenosine monophosphate ..... cAMP
Direct pathway MSN D1-MSN
Dopamine(rgic) ..... DA
Dorsolateral striatum ..... DM
Dorsomedial striatum ..... DL
Enhanced yellow fluorescent protein ..... EYFP
Excitatory post-synaptic potential ..... EPSP
Fast spiking interneuron ..... FSI
Gamma-aminobutyric acid ..... GABA
High frequency stimulation ..... HFS
Indirect pathway MSN D2-MSN
Inwardly rectifying potassium channel ..... Kir
Ionotropic GABA receptor ..... $GABA_A$
Kappa opioid receptor ..... KOR
Long term depression ..... LTD
Long term potentiation ..... LTP
M1-type acetylcholine receptor ..... M1 AChR
Medium spiny neuron ..... MSN
Metabotropic GABA receptor ..... $GABA_B$
Metabotropic glutamate receptor ..... mGluR
NMDA receptor subunit NR2A
NMDA receptor subunit NR2B
NMDA receptor ..... NMDAR

N-methyl-D-aspartate ..... NMDA
Rheobase ..... Rheo
Small conductance potassium channel..... SK
Spike timing dependent plasticity ..... STDP
Voltage gated calcium channel ..... VGCC

ABSTRACT

THETA-BURST LTP IN THE DORSAL STRIATUM: ITS DEVELOPMENTS AND USE TO ILLUMINATE STRIATAL DYNAMICS UNDERLYING SKILL LEARNING, AND THE ABERRANT LEARNING BEHIND ADDICTION.

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George Mason University, 2015

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Corticostriatal plasticity facilitates action selection and skill learning through dynamic enhancement ("long term potentiation" or LTP) and reduction ("long term depression" or LTD) in communication strength between neurons. Striatal primary neurons are divided into two classes: motor-enhancing "direct" and motor-suppressing "indirect" pathway neurons. The regulation of plasticity in these two classes is critical because pathway imbalance is a noted feature in Parkinson's disease, and strong class-specific plasticity accompanies exposure to drugs of abuse. Thus, it is important to understand striatal plasticity not only to identify neural learning mechanisms, but also because dysregulation of plasticity processes serving learning contributes to disease states.

Dorsal striatal LTP has been difficult to induce in brain slices without resorting to unrealistic electrical or chemical treatments. The **first research aim** is to develop a striatal LTP induction paradigm that resembles brain activity observed during learning behavior. I achieve this by developing a theta-burst stimulation (TBS) protocol modeled after *in vivo* striatal activity during learning. I show the evoked LTP is indeed reliant on kinases and neurotransmitter receptors implicated in learning. This is a powerful tool for any researcher interested in recreating naturalistic striatal plasticity in acute brain slice.

The **second research aim** within this dissertation is to clarify the relationship between striatal plasticity and learning behavior. Prior works show a transition in the engaged dorsal striatal subregion as skill performance shifts from an attentive phase to a more habitual phase. In addition, increased striatal activity in one hemisphere is known to generate contralateral turning behavior. Thus, I analyze striatal subregional plasticity at different time points as animals learn to execute a consistently rewarded T-maze turn, and further characterize lateralized striatal plasticity as animals are trained to turn. I find that modifications in evoked plasticity and in intrinsic neuronal excitability differ between hemispheres relative to the direction of the trained turn. More significantly, I find that striatal LTP and LTD are independently modulated during learning rather than reciprocally related as previously suggested. Finally, analysis of neuronal morphology reveals novel dendritic pruning in trained animals, without a change in spine density. This dendritic pruning may enhance signal to noise ratio of information transmission through the striatum.

The **third research aim** within this dissertation is to identify the striatal pathway(s) expressing LTP, and neuromodulatory roles in induction. Direct and indirect pathway neurons co-release distinct neuropeptides, including opioids known to influence motivational and addictive states. Whether LTP is naturally expressed in both pathways is not known, and little is known about potential intra-striatal pathway interaction via the co-released neuropeptides. Recordings from single striatal neurons suggest that both pathways express LTP. By genetically expressing channel rhodopsin in either pathway to elevate pathway-specific co-release during TBS LTP induction, we identify a mechanism whereby direct pathway neurons suppress corticostriatal LTP, possibly via reduced intra-striatal dopamine release.

In summary, the work comprising this dissertation furthers the field of striatal learning and plasticity by supplying a robust, physiological LTP induction method, and by using this new method to demonstrate altered striatal plasticity consequent to striatal dependent learning. Finally, revealing LTP modulation by endogenous opioids has major implications for understanding the aberrant learning involved in addiction to drugs of abuse.

1. OVERVIEW OF THE STRIATUM

The striatum is the primary input nucleus of the basal ganglia, and regulation of information flow through the striatum is important in learning, performing, and maintaining new adaptive behaviors. Information flow is controlled by network dynamics which influence and are controlled by striatal plasticity; thus, understanding this interaction is critical not only to understand the biology behind learning, but also because misdirected or impaired striatal dynamics have serious clinical ramifications. Striatal plasticity is pathologically usurped by substances of abuse to foster addictive states. For instance, dorsal striatal plasticity enables learning to associate environmental cues, such as a light or tone signaling reward availability, with a rewarded behavior, such as lever pressing for food or drug reward (Vanderschuren et al., 2005). Once learned, this type of cued action-selection is difficult to reverse and is rapidly re-acquired following extinction, accommodating drug addiction and relapse behavior (Balleine and O'Doherty, 2010). Striatal dysregulation also contributes to the motor and cognitive deficits seen in Parkinson's disease and dyskinesia. Striatal function is disrupted by the loss of the striatally-enriched neuromodulator dopamine in Parkinson’s disease. Parkinson's disease patients become progressively akinetic, and also demonstrate abnormal learning (Ghilardi et al., 2003;Levin and Katzen, 2005). In animal models of Parkinson's disease, some forms of striatal plasticity may be lost entirely (Calabresi et al., 1997;Shen et al.,

2008;Paille et al., 2010), and similar plasticity is abnormally persistent in animals showing dyskinesia resulting from levodopa, the most common clinical treatment for Parkinson's disease (Bagetta et al., 2010;Calabresi et al., 2000a;Picconi et al., 2003). The research within this dissertation focuses on dorsal striatal plasticity in healthy learning because identifying the natural capacities and dynamics of a system will let scientists identify disorder by contrast, and lead researchers designing new therapies to recognize, protect and restore healthy neural dynamics.

Throughout all striatal subregions, the striatal medium spiny neurons (MSNs) comprise approximately ninety five percent of all neurons, and synapses onto MSNs integrate glutamatergic afferents from a variety of cortical regions as well as from thalamus. Cortical regions supplying dorsal striatum include visual, associative, limbic, somatosensory, and motor cortex. In addition, afferents from substantia nigra pars compacta (SNc) supply abundant intra-striatal dopamine, which is an important neuromodulator throughout the striatum and is released in response to and in anticipation of reward (Bermudez and Schultz, 2014). Following intra-striatal afferent integration onto MSNs, these GABAergic projection neurons exit dorsal striatum to synapse on additional basal ganglia nuclei which ultimately feedback on to cortex and thalamus (Figure 1). The MSNs are divided between two classes with opposite effects on motor behavior. MSNs comprising the "direct pathway" are known as D1-MSNs for their G_{olf}-coupled D1-type dopamine receptors, and selective optogenetic activation of this population of neurons *in vivo* promotes locomotion (Kravitz et al., 2010). Direct pathway efferents project to the internal segment of the globus pallidus (GPi), thereby inhibiting

GPi GABAergic control over thalamus. Thus the D1-MSNs and direct pathway disinhibit the downstream thalamus and cortex, and are generally credited with facilitating action (Figure 1). MSNs comprising the "indirect pathway" are known as D2-MSNs for their $G_{i/o}$-coupled D2-type dopamine receptors, and selective optogenetic activation of this population of neurons *in vivo* elicits freezing behavior (Kravitz et al., 2010). Indirect pathway efferents are GABAergic projections to the external segment of the globus pallidus (GPe). These relieve GPe inhibition of the GPi, which exerts greater GABAergic influence over thalamus as a result. The D2-MSNs and indirect pathway inhibit the downstream thalamus and cortex, and are generally credited with inhibiting action (Figure 1).

Figure 1.

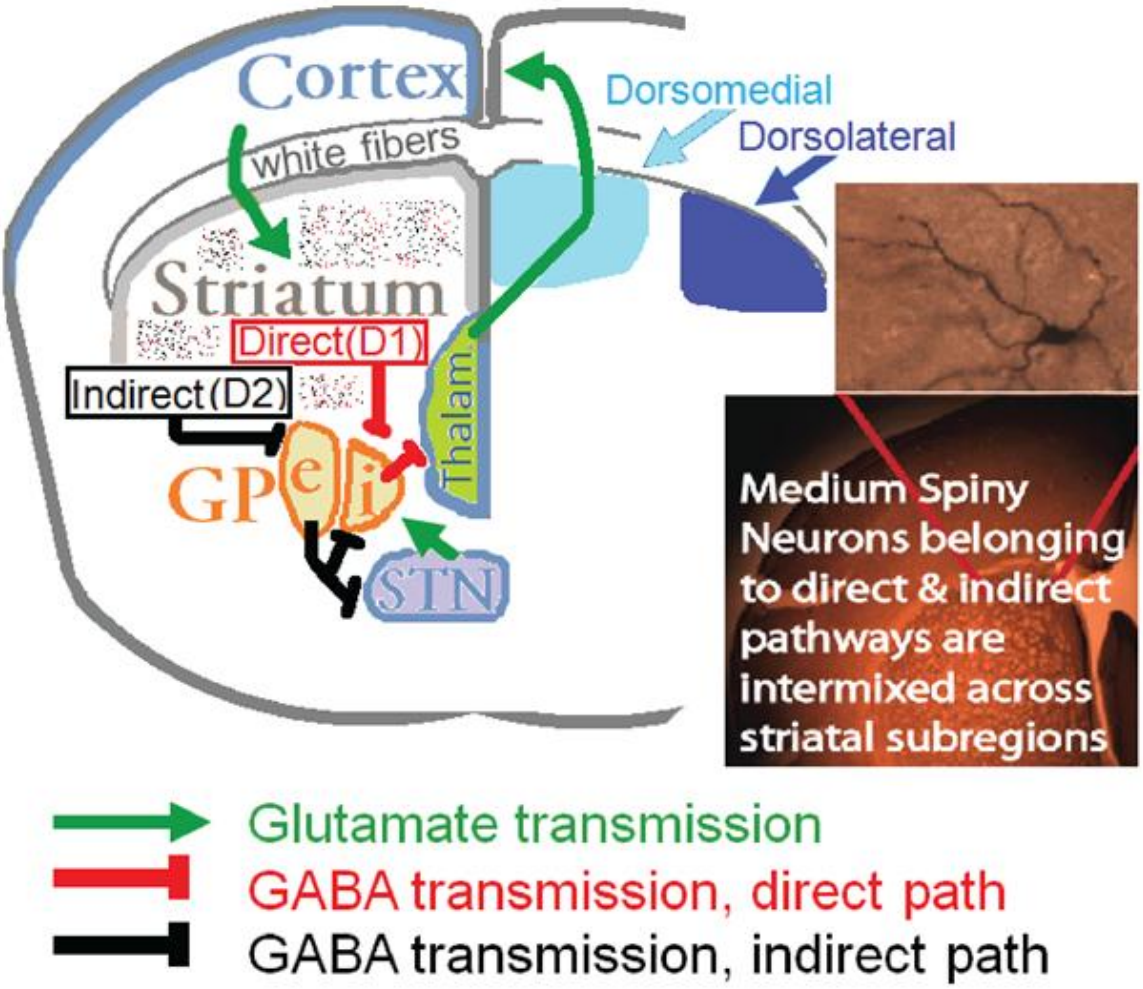


Figure 1. Circuits and subregions in dorsal striatum. Direct pathway (in red, D1-MSNs) and indirect pathway (in black, D2-MSNs) projections are diagramed at left. GPe/i = globus pallidus external/ internal, respectively. STN = subthalamic nucleus. Thalam. = thalamus. Dorsomedial and dorsolateral subregions are diagramed at upper right. Lower right images show a single biocytin-filled MSN at 40x magnification, coming from the dorsomedial subregion of a coronal mouse brain slice.

Learning and Plasticity

The striatum, at the intersection of limbic, motor and reward systems, is perfectly situated to evaluate action and to modify animal behavior. Dorsal striatum is critical for instrumental learning (Yin et al., 2005;Graybiel, 1995), motor skill development (Yin et al., 2009), cued action-selection (Packard and Teather, 1997), and habit formation (Yin and Knowlton, 2006). Learning types and stages can be distinguished behaviorally by differences in the type of information animals use to direct decision making, and by differences in the stability of the learned behavior. For instance the realization of a new contingency and the performance of a newly learned task require broad attention as the most relevant information has not yet been identified. Skill acquisition is fastest when salient and reliable cues provide this information, whereas complex or variable contingencies slow skill acquisition and preserve flexibility in animal behavior (Derusso et al., 2010;Gardner et al., 2013). Rapid performance of the same task after extensive training may result, in part, from honing attention to a reduced cue set. This is illustrated by stages of learning performance on the T-maze. T-maze training rewards animals for making a consistent turn to locate food. After only a brief period of T-maze training, animals started from a novel position on the maze demonstrate behavioral flexibility and attention to extra-maze cues by committing a novel body turn to navigate to the spatial location rewarded in training. After extensive T-maze training, animals started from a novel position on the maze will perform the same right or left body turn practiced in training; this suggests attention has shifted away from extra-maze cues and that behavior has become more automatic, with the animal performing immediate egocentric body

turns. Ultimately, over-trained performance can become habitual, or insensitive to new and salient information from the environment. Learned performance has become habitual rather than goal-directed when the behavior is maintained despite reward devaluation (Hogarth et al., 2013). If a reward is devalued or the reward contingency changes, goal directed animals more readily amend their behavior to satisfy the new contingency at early stages of training, whereas habitually responding animals will continue performing according to the old contingency (Hilario et al., 2007).

Distinct neural substrates serve these different types and stages of learning. In dorsal striatum, dorsomedial and dorsolateral subregions (Figure 1) serve dissociable aspects of skill learning (Ragozzino, 2003;Murray et al., 2012). The dorsomedial striatum is engaged in periods of flexibility in decision-making and serves early learning, while the dorsolateral striatum streamlines practiced skills and habits later in learning. This shift in striatal engagement is indicated by changes in behavior following subregional lesions (Yin et al., 2004;Lee et al., 2014;Whishaw et al., 1987), *in vivo* neural activity (Thorn and Graybiel, 2014;Yin et al., 2009), and changes in glutamate receptors suggestive of synaptic plasticity (Kent et al., 2013;Yin et al., 2009;Shan et al., 2014).

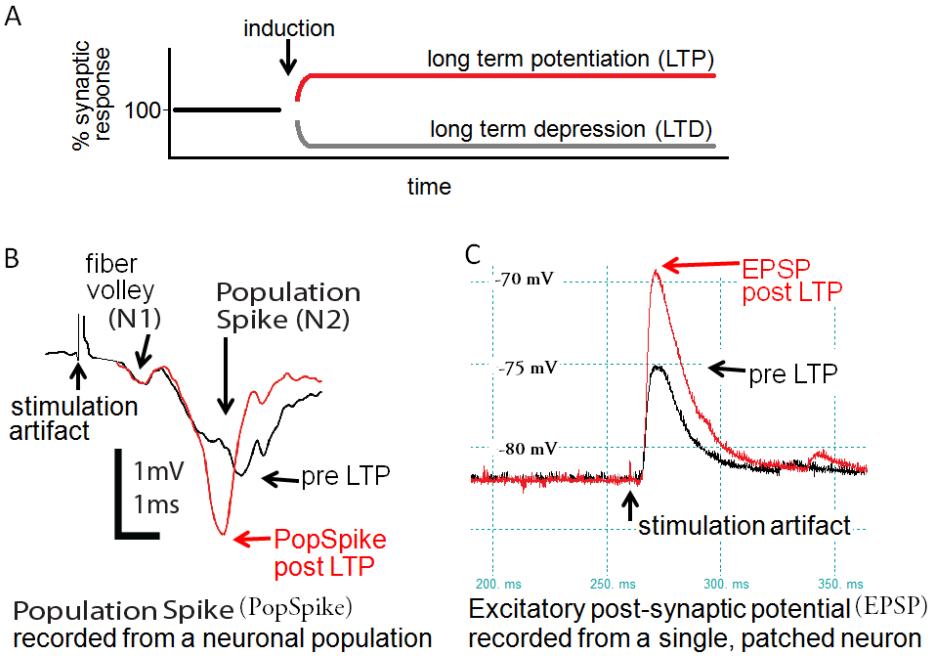
In addition to serving different roles in learning process, dorsomedial and dorsolateral MSNs demonstrate behavior-related coherence to subtly different frequencies during learning (Thorn and Graybiel, 2014). MSNs fire below 5 Hz on average *in vivo*, with maximum spontaneous firing occurring in short bursts not higher than 50 Hz (Barnes et al., 2005;Miller et al., 2008). Despite infrequent firing on the part of individual MSNs, dorsal striatal local field potentials demonstrate MSN population

coherence around theta frequencies (7-11 Hz) during learning behavior. Coherent theta rhythms are modulated in an activity-dependent manner during learning (Buzsaki, 2005;Koralek et al., 2012;Tort et al., 2008). Specifically, dorsal striatal MSN firing dynamically aligns to theta rhythms during salient task points such as initiation, completion, and decision-making moments (Tort et al., 2008;DeCoteau et al., 2007). Dorsomedial MSNs demonstrate coherence to higher theta frequencies (~10Hz) whereas dorsolateral MSNs demonstrate coherence to lower theta frequencies (~5Hz) during learning (Thorn and Graybiel, 2014). This striatal subregional difference in learning-related MSN coherence may be causally linked to subregional difference in striatal behavioral support, and also to subregional difference in the striatal network dynamics directing plasticity.

Experimental evidence supports the role of activity dependent change in synaptic strength, known as synaptic plasticity, in dorsal striatum as a substrate for learning. *In vivo* and behavioral observations (Shen et al., 2011;Pascoli et al., 2012) indicate that shifts in synaptic weight underlie certain forms of learning and memory. There are many modes of neural plasticity, both different types of synaptic plasticity and plasticity of other neuron characteristics. Lasting enhancement in synaptic communication between neurons is called long term potentiation (LTP) (Figure 2). *In vivo* stimulation of cortical or SNc afferents leads to corticostriatal LTP, and the strength of this LTP correlates with learning speed (Charpier et al., 1999;Reynolds et al., 2001). Changes in long term depression (LTD), i.e. the down regulation of communication strength between neurons, are observed *ex vivo* following skill learning (Yin et al., 2009). In addition to synaptic

modification, changes in neuronal excitability and morphology may serve learning. In dorsal striatum, potassium channel regulation accompanies spatial learning (Truchet et al., 2012), and modifies the extent to which synaptic plasticity can be evoked (Nazzaro et al., 2012). Dendritic spine growth is cited as an indication of LTP in cortical regions (Kasai et al., 2010), while a recent work shows that memory and LTP are supported by spine loss in behaviorally-engaged hippocampal circuits, suggesting signal to noise enhancement through synaptic pruning (Sanders et al., 2012). Within striatum, any of these plasticity modes could contribute to allow experience to selectively enhance critical action-outcome or stimulus-response associations, resulting in learning.

Figure 2.



Population Spike (PopSpike) recorded from a neuronal population

Excitatory post-synaptic potential (EPSP) recorded from a single, patched neuron

Figure 2. Synaptic plasticity is activity-dependent adjustment in synaptic response amplitude. **A.** Enhancement (in red, LTP) and reduction (in grey, LTD) in synaptic responsiveness. **B.** Example traces before (black) and after (red) LTP induction, recorded extracellularly from a population of striatal neurons. **C.** Example traces before (black) and after (red) LTP induction, recorded intracellularly from a single MSN.

Differences between MSN subtypes

Both D1- and D2-MSNs are interspersed throughout the dorsal striatum, and receive similar afferent inputs in terms of the composition and timing of afferent signaling, including glutamate from cortex and thalamus, dopamine from SNc, and acetylcholine from interneurons. In addition, inhibitory interactions between neurons of the same class and between different classes, together with inhibitory input to both classes from a common pool of fast spiking interneurons, creates a common network environment and maintains more-or-less balanced activity between the D1- and D2-MSNs (Damodaran et al., 2014). It is postulated that plasticity refines the balanced activity between these MSN classes to shape and maintain learned behavior. Importantly, this common network environment may be differentially interpreted by class differences in receptor expression profiles which could in turn lead to distinct plasticity responses in either MSN class. Both classes express either G_{olf} or G_s –coupled receptors which increase adenylyl cyclase, elevating cAMP and activating PKA (a kinase implicated in LTP and learning). In addition, both classes express $G_{i/o}$-coupled receptors, which reduce cAMP. Finally both classes express G_q-coupled receptors, which increase PLC, intracellular calcium, and can lead to production of endocannabinoids (required for LTD) or PKC (a kinase implicated in LTP and learning). However the same neurotransmitter

encounters opposite G-protein coupling on D1- versus D2-MSNs (Figure 3A). D1-MSNs respond to dopamine with G_{olf} –signaling, and respond to adenosine with $G_{i/o}$-signaling. D2-MSNs respond to dopamine with $G_{i/o}$ –signaling, and respond to adenosine with $G_{s/olf}$-signaling. Both classes respond to acetylcholine with G_q-signaling, but only D1-MSNs additionally respond with G_i-signaling. These differences in receptor coupling between the classes mean that the same extracellular milieu of transmitters may have opposite effects on the intracellular LTP effector PKA, for example, in a D1- versus a D2-cell. This is a possible mechanism for the mixed MSN population to respond to the same stimulus by expressing LTP in one cell class while the other expresses LTD or no change.

Differences in *ex vivo* plasticity between D1- and D2-MSN populations have been noted (Mathur et al., 2013;Bateup et al., 2010;Pascoli et al., 2012;Valjent et al., 2010;Centonze et al., 2001;Kreitzer and Malenka, 2007;Yin et al., 2009). Others find no difference in plasticity between MSNs (Wang et al., 2006;Bagetta et al., 2011;Pawlak and Kerr, 2008) and some of the reported differences may be artifacts of transgene expression systems (Shen et al., 2008;Kramer et al., 2011). A recent paper shows both D1- and D2-MSNs express corticostriatal LTD in response to a common induction, but that dopamine oppositely modulates LTD expression in either class (Wu et al., 2015). Marked difference in plasticity or activity between cell classes is associated with pathologies of dyskinesia and addiction. In mice with L-DOPA induced dyskinesia, overly strong activity in the PKA pathway leads to excessive LTP (Picconi et al., 2003), and ERK is elevated exclusively in D1-MSNs (Santini et al., 2009). Therefore LTP restriction by MSN class may be a pathological phenomenon, either causal of symptomatic of dyskinesia. Drug

challenge with cocaine or heroin activates PKA and PKC targets preferentially in D1-MSNs (Xie et al., 2010;Tropea et al., 2008;Pascoli et al., 2012), and drug sensitization-related LTP is restricted to D1-MSNs (Pascoli et al., 2012). Whether the imbalance seen in plasticity-related kinase activity across cell class serves LTP in the absence of drugs is not clear. This imbalance in pathway activation may also be pathological. It is also possible that restriction of LTP to one MSN class may occur naturally, and simply becomes more pronounced with the extreme reinforcement provided by drug challenge.

The opposite influence either cell class exerts on motor behavior might be leveraged to serve motor skill learning through LTP in either class, though differential receptor expression profiles suggest the ideal environment for plasticity induction differs between classes (Wu et al., 2015). As described above, both MSN classes have the means to activate kinases serving LTP. As illustrated in Figure 3, both are equipped to activate the learning-associated kinase ERK (Huang et al., 2010), since both contain G_q–coupled receptors to activate PKC (Perez-Burgos et al., 2008;Kim et al., 2013;Gubellini et al., 2004) and G_s-coupled receptors to activate PKA (Higley and Sabatini, 2010). Since it is not known whether both MSN classes naturally express LTP or in what behavioral context, it is important to identify the network conditions eliciting plasticity in both classes, and the factors influencing these conditions.

Aside from the constant difference in receptor coupling distinguishing MSNs, D1- and D2-MSN axon collaterals terminating within the striatum might actively influence network conditions locally by the co-release of class-specific neuropeptides (Figure 3B). D1-MSNs release dynorphin, and D2-MSNs release enkephalin, both of which act via

presynaptic μ-opioid receptors to depress glutamate release (Atwood et al., 2014;Blomeley and Bracci, 2011). However μ-opioid receptors have much higher affinity for enkephalin (Wee and Koob, 2010), giving D2-MSNs stronger influence over μ-opioid receptors. In contrast, dynorphin released from D1-MSNs has greater affinity for the dedicated Kappa opioid receptors, and has negligible influence on glutamate release dorsomedially (Atwood et al., 2014). Kappa opioid receptors are located presynaptically at nigrostriatal terminal within striatum (Bruijnzeel, 2009) where they permit dynorphin to regulate release of dopamine. The Kappa opioid receptors maintain dopaminergic terminals at rest, reduce dopamine release, and speed dopamine re-uptake (Kivell et al., 2014a). This supplies D1-MSNs with a mechanism to reduce the intra-striatal release of a critical plasticity modulator, which exerts opposite influence over D1- and D2-MSNs through the differential dopamine receptor expression described above. D1-MSNs also release substance P, which acts at NK1 receptors presynaptically on glutamate afferents and has been shown capable of facilitating glutamate release onto surrounding MSNs (Blomeley et al., 2009). These differences supply potential mechanisms for cell class specific roles in the induction of plasticity.

Figure 3.

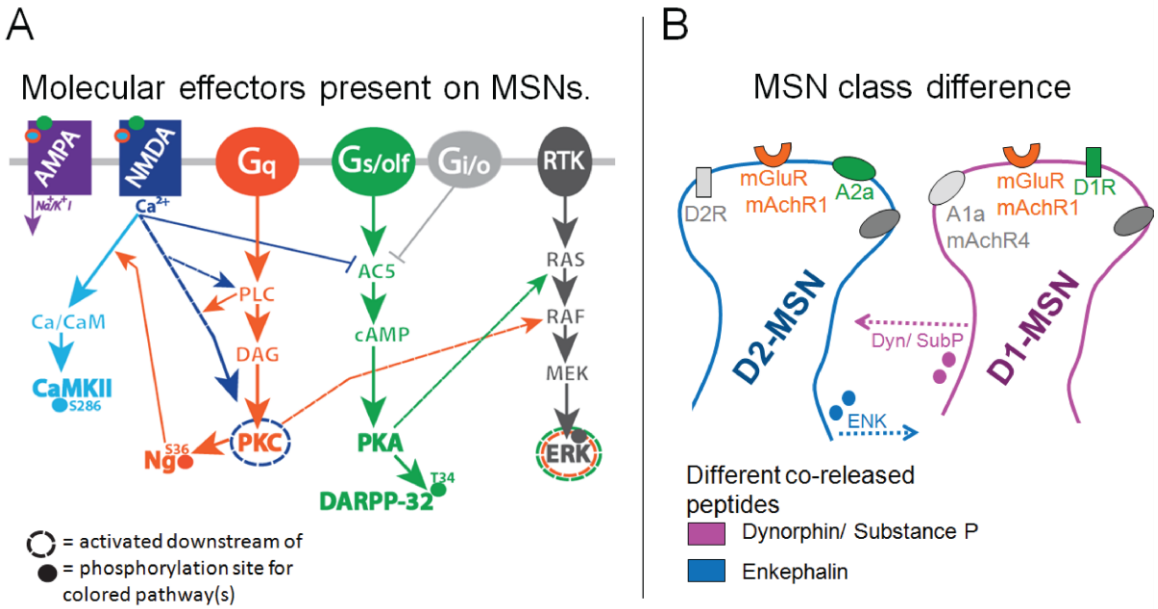


Figure 3. Molecular effectors relevant to learning and plasticityare expressed on MSNs. **A.** Ionotropic glutamate receptors and numerous metabotropic Gq-coupled pathways exist on both D1- and D2-MSNs. **B.** Common intracellular pathways are activated in response to dissimilar ligand-binding between D1- and D2-MSNs. In addition to GABA, the different MSN populations co-release different neuro-active peptides within the striatum.

Together the integration of cortical, thalamic and dopaminergic afferents through dorsal striatal plasticity directs addiction and disease development as well as serving healthy learning. This dissertation builds on existing literature reflecting learning related activity *in vivo* with the design of an *ex vivo* LTP model in Chapter two. Knowledge of subregional striatal roles in learning is expanded by results in Chapter three which investigates the distribution of synaptic, intrinsic and morphological plasticity with learning. Finally in Chapter four, new direct and indirect pathway differences in LTP expression and modulation are demonstrated.

Dissertation goals and significance

The specific gaps in knowledge within the field which we aimed to address are described below in connection with a brief description the progress each subsequent chapter makes in bridging these gaps.

Chapter 2 goals and significance

Given the accepted role of the dorsal striatum in learning, and the *in vivo* evidence for corticostriatal LTP during learning, dissecting neural plasticity mechanisms for striatal learning would be greatly facilitated by *ex vivo* studies of LTP. However it is important to keep in mind that *ex vivo* LTP can result from a variety of dissimilar induction methods, and this can result in dissimilar intracellular processes leading to LTP (Malenka and Bear, 2004;Kim et al., 2010). For instance, striatal LTP may be evoked through bath application of a drug, through hypoxic treatment, and through high frequency electrical stimulation of afferent fibers. The LTP evoked in these three cases may be completely dissimilar in terms of the engaged intracellular cascades and resultant cellular modification. Within dorsal striatum, the ability to study LTP serving learning has been limited by existing induction mechanisms. Chemical LTP has been valuable for demonstrating capacities of the system (Spencer and Murphy, 2002), but not for modeling the system's response to cortical input. Unlike in the hippocampus, high frequency stimulation (HFS) has been used to evoke corticostriatal LTP (Centonze et al., 1999;Partridge et al., 2000) but not regularly. This is because corticostriatal HFS LTP is

not reliably obtained between labs or studies. Corticostriatal HFS LTP is made reliable by bathing tissue in Mg^{2+}-free solution (Picconi et al., 2003;Calabresi et al., 1992b;Jia et al., 2010) to eliminate voltage-dependence of NMDA receptors. However, this approach introduces a possible disconnect between synaptic activity and spatiotemporal calcium dynamics. A physiologically realistic LTP induction protocol for dorsal striatum to reliably reproduce subcellular mechanisms underlying learning behavior *in vitro* would be a significant benefit to the field.

Our goal was to facilitate the study of striatal learning processes by developing a method to evoke *ex vivo* plasticity which is mechanistically similar to that evoked in behaving animals. In both dorsal striatum and in hippocampus, correlation between neuron firing and theta rhythm is enhanced at salient task points such as initiation, completion, and decision-making moments (DeCoteau et al., 2007;Tort et al., 2008). The significance of theta-burst stimulation (TBS) for determining memory-relevant plasticity has been demonstrated in the hippocampus. Strength of hippocampal theta rhythm increases with successful learning and recall (Robbe and Buzsaki, 2009). Hippocampal TBS but not HFS LTP shares characteristics with memory such as reliance on PKA and protein synthesis (Abel et al., 1997;Staubli et al., 1999). We pursued the idea that native temporal sensitivity may be the key to inducing physiologically plausible *ex vivo* LTP in the striatum as well.

Progress in understanding dorsal striatal plasticity was impeded for years by difficulty evoking *ex vivo* corticostriatal LTP reliably without resorting to unnatural stimulation paradigms. Chapter two of this dissertation addresses this issue by leveraging

native temporal sensitivity of the system to evoke striatal LTP in the presence of physiological levels of Mg^{2+}. To facilitate application in behavioral studies in which adult animals are more stable subjects, adult mice are used. A striatal TBS protocol with high fidelity to learning behavior is developed. LTP is shown to rely both on learning-related afferent timing, and on learning-related receptors and kinases. As a result of publishing this work, a convincing *ex vivo* model for striatal plasticity serving memory and motor adaptation is currently available for all striatal researchers, fulfilling a critical need in the study of striatal-based learning.

Chapter 3 goals and significance

Both lesions and in vivo recordings demonstrate a transition in the engaged dorsal striatal subregion - from dorsomedial to dorsolateral - as skill performance shifts from an attentive phase to a more automatic or habitual phase. T-maze training transitions rats from attentive, action-outcome to automatic, stimulus-response performance (Packard, 1999;Yin and Knowlton, 2004), and specifically reinforces turning behavior. As demonstrated by hemispheric lesion and *in vivo* recordings, turning behavior corresponds to increased striatal activity in the contralateral hemisphere (Ungerstedt et al., 1969;Cui et al., 2013). However the development of plasticity sculpting a learned turn is uncharacterized. The only study predating this dissertation to demonstrate learning-related change in *ex vivo* plasticity across striatal subregions investigates animals trained on a symmetrical rotarod motor skill (Yin et al., 2009). This study reports enhanced long-term depression (LTD) and altered AMPA:NMDA ratios in the dorsolateral striatum of

extensively-trained animals (Yin et al., 2009). One interpretation for this result is that recent LTP elevates synaptic weight, thereby enhancing room for synaptic weight change in the opposing direction, i.e. LTD (Cooper and Bear, 2012;Lin, 2010). Alternatively, learning may modulate LTD and LTP independently rather than reciprocally. Distinguishing these possibilities requires direct comparison of bidirectional plasticity.

Chapter three of this dissertation addresses this question by testing anatomical distribution of evoked bidirectional striatal plasticity as animals transition from early, attentive *place* to late, habitual *response* strategies with T-maze learning (Packard, 1999). To pursue this broad goal, we utilized a lateralized T-maze skill so that we are able to separate analysis of striatal physiology over time, performance strategy, and also turn-relative hemisphere in the context of lateralized learning. To address whether plasticity in intrinsic excitability or neuron morphology may serve striatal learning, we measure excitability and morphology of striatal medium spiny neurons in parallel with synaptic plasticity measures. We also sought to address whether striatal plasticity underlies dissociable cognitive strategies, or more purely reflects difference in locomotor performance.

Research in Chapter three of this dissertation is the first direct comparison of bidirectional plasticity in striatal brain slices, the first evaluation of striatal plasticity by hemisphere relative to a learned turn, and the first demonstration of intrinsic and morphological plasticity in concert with T-maze learning. We find that long-term potentiation and depression (LTP and LTD) are independently modulated with learning rather than reciprocally linked as previously suggested. Our results establish that

modulation of evoked synaptic plasticity with learning depends on striatal subregion, training stage, and hemisphere relative to the learned turn direction. Neuronal reconstructions indicate dendritic remodeling after training which may represent a novel form of pruning. In conclusion, we newly describe region- and hemisphere-specific changes in striatal synaptic, intrinsic, and morphological plasticity corresponding to T-maze learning.

Chapter 4 goals and significance

Differential expression of plasticity by either MSN class could be important for plasticity refining behavior because of cell class difference in locomotor influence: D1-MSNs comprise the putatively motor-enhancing direct pathway and D2-MSNs comprise the putatively motor suppressing indirect pathway. As described above, both populations are equipped to engage LTP machinery (i.e. supportive kinases). However, distinct post synaptic receptor coupling to common intracellular cascades means that these pathways may be engaged by distinct means in either class. Whether LTP is evoked by TBS in both D1- and D2-MSNs is not known. It is clear that corticostriatal plasticity results from convergent glutamatergic and dopaminergic afferents. However, plasticity might also be modulated by MSN axon collaterals which terminate within the striatum and release different neuromodulatory peptides onto neighboring MSNs. D1-MSNs co-release the neuropeptide Substance P, which acts at NK1 receptors to facilitate glutamate release primarily on to D2-MSNs during burst firing (Blomeley and Bracci, 2008;Jakab et al., 1996;Blomeley et al., 2009). Therefore, Substance P release from D1-MSNs during TBS

may facilitate corticostriatal transmission at D2-MSNs. D1-MSNs also co-release the opioid dynorphin, which suppresses intra-striatal dopamine release through its binding at presynaptic kappa opioid receptors on dopaminergic afferent terminals. Reduced dopamine within the striatum could differentially influence corticostriatal plasticity at D1- and D2-MSNs due to the differential dopamine receptor coupling described above. D2-MSNs co-release the opioid enkephalin, which suppresses corticostriatal glutamate release through its binding at presynaptic mu and delta opioid receptors on cortical and thalamic afferents (Bruijnzeel, 2009;Atwood et al., 2014). Difference in co-release from MSN axonal collaterals onto fellow MSNs within the striatum represents the potential for pathway difference in the control of corticostriatal plasticity.

My goals in Chapter four of this dissertation are to identify the cell class expressing LTP in response to TBS, and also to identify the influence of either direct or indirect pathway co-release on the induction of LTP in the potentiating MSNs. To pursue these questions, whole-cell LTP experiments are performed *ex vivo* in tissue from mice expressing channel rhodopsin in D1-MSNs, and separately in mice expressing channel rhodopsin in D2-MSNs. In either mouse line, the identity of patched neurons is established by depolarization in response to light. The influence of endogenously released, pathway-specific neuropeptides on corticostriatal LTP is amplified by using light to activate channel rhodopsin expressed either on D1- or else on D2-MSNs during TBS, encouraging cell class specific action potentials and co-release. Potential mechanisms for plasticity modulation are investigated pharmacologically.

The research in Chapter four of this dissertation provides new information on pathway-specific roles in dorsal striatal LTP. Our experiments indicate that LTP occurs in response to TBS in both D1- and D2-MSNs. We discover that co-release from the direct pathway suppresses TBS LTP onto both D1- and D2-MSNs. We show that this regulation of LTP by MSNs requires activity of Kappa opioid receptors by D1-MSN co-released dynorphin. Our findings suggest that LTP-like changes that develop with addictive behavior and appear to be restricted to D1-MSNs, as discussed above, are pathological in their pathway restriction since we find evidence for TBS LTP in both pathways in healthy animals. Kappa opioid receptor agonists have been investigated as a potential tool in combating addiction due to their regulation of dopamine release (Schlosburg et al., 2013;Walker and Koob, 2008). Our results affirm that this is an effective and physiologically relevant target, given the potential of endogenous dynorphin release to constrain striatal LTP by engaging the Kappa opioid receptor.

In summary, this dissertation research yields new insight into physiological conditions eliciting corticostriatal LTP, describes the appearance and distribution of plasticity throughout maze learning, addresses whether both MSN classes express LTP, and identifies a mechanism whereby direct pathway MSNs modulate LTP induction.

2. SENSITIVITY TO THETA-BURST TIMING PERMITS LTP IN DORSAL STRIATAL ADULT BRAIN SLICE

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Abstract

Long term potentiation (LTP) of excitatory afferents to the dorsal striatum likely occurs with learning to encode new skills and habits, yet corticostriatal LTP is challenging to evoke reliably in brain slice under physiological conditions. Here we test the hypothesis that stimulating striatal afferents with theta-burst timing, similar to recently reported *in vivo* temporal patterns corresponding to learning, evokes LTP. Recording from adult mouse brain slice extracellularly in 1mM Mg^{2+}, we find LTP in dorsomedial and dorsolateral striatum is preferentially evoked by certain theta-burst patterns. In particular, we demonstrate that greater LTP is produced using moderate intra-

burst and high theta-range frequencies, and that pauses separating bursts of stimuli are critical for LTP induction. By altering temporal pattern alone, we illustrate the importance of burst-patterning for LTP induction, and demonstrate that corticostriatal long term depression is evoked in the same preparation. In accord with prior studies, LTP is greatest in dorsomedial striatum and relies on NMDA receptors. We also demonstrate a requirement for both G_q- and $G_{s/olf}$-coupled pathways, as well as several kinases associated with memory storage: PKC, PKA, and ERK. Our data builds on previous reports of activity-directed plasticity by identifying effective values for distinct temporal parameters in variants of theta-burst LTP induction paradigms. We conclude that those variants which best match reports of striatal activity during learning behavior are most successful in evoking dorsal striatal LTP in adult brain slice without altering ACSF. Future application of this approach will enable diverse investigations of plasticity serving striatal-based learning.

Introduction

The dorsal striatum hosts an intersection of cognitive, limbic, motor and reward systems which combine to impart neural changes underlying learning. Striatal activity is critical for instrumental learning (Yin et al., 2005;Graybiel, 1995), motor skill development (Yin et al., 2009), cued action-selection (Packard and Teather, 1997), and habit formation (Yin and Knowlton, 2006). Distinct types and stages of learning differ in their engagement of medial and lateral dorsal striatal regions (Yin et al., 2006;Pauli et al., 2012). Observations *in vivo* and *ex vivo* suggest that changes in strength of connection

between neurons underlie striatal learning and memory (Yin et al., 2009;Koralek et al., 2012;Shen et al., 2011;Pascoli et al., 2011;Pauli et al., 2012). Stimulation of glutamatergic and dopaminergic afferents to dorsal striatum leads to *in vivo* corticostriatal long term potentiation (LTP), the strength of which correlates with learning speed (Charpier et al., 1999;Reynolds et al., 2001). Rotarod training reduces long term depression (LTD) *ex vivo* in the dorsal striatum (Yin et al., 2009). Despite its importance in learning and memory, investigation of mechanisms underlying information storage in dorsal striatum has been limited by the difficulty in evoking reliable, long lasting LTP in striatal brain slice under physiological conditions.

Consistency in predictably evoking unidirectional plasticity (either LTP or LTD) *ex vivo* is no doubt complicated by regional variation in striatal tissue composition mixed with disparity in experimental approach (Reynolds and Wickens, 2002;Kreitzer and Malenka, 2008). Two generally consistent approaches taken to evoke activity-dependent *ex vivo* corticostriatal plasticity are high frequency stimulation (HFS) and spike-timing dependent plasticity (STDP). Corticostriatal HFS typically evokes LTD in normal Mg2+ ACSF (Lovinger et al., 1993b;Walsh, 1993;Xia et al., 2006;Kreitzer and Malenka, 2005), though it has also been reported to evoke plasticity of mixed direction (Akopian et al., 2000;Akopian and Walsh, 2006;Spencer and Murphy, 2000). The same HFS reliably evokes corticostriatal LTP in the absence of Mg2+, an ion natively conveying NMDA receptor voltage-dependence (Fino et al., 2005;Guan et al., 2010;Calabresi et al., 1992b;Arbuthnott et al., 2000;Kerr and Wickens, 2001). Drawbacks to HFS include use of unrealistically high frequencies for striatum, and use of 0 Mg^{2+} which nullifies NMDA

receptors as voltage-sensitive coincidence detectors, potentially blunting temporal sensitivity in calcium influx. Spike-timing dependent plasticity protocols employ lower, more reasonable frequencies to pair postsynaptic action potentials with precisely timed presynaptic stimulation in normal Mg2+, and in these regards STDP is more physiological than HFS. STDP can be used to evoke LTP or LTD as desired based on the relative timing of activity across the synapse (Fino et al., 2005) and whether $GABA_A$ receptors are blocked (Fino et al., 2010;Paille et al., 2013), but as with HFS stimulation, STDP LTP is not consistently observed (Shindou et al., 2011). Because diverse induction paradigms invoke plasticity by way of distinct molecular mechanisms (Asrar et al., 2009;Lerner and Kreitzer, 2012;Petersen et al., 2003;Ronesi and Lovinger, 2004), it would be ideal to use normal magnesium solutions in combination with physiological, learning-like activity to induce long lasting plasticity for the purpose of examining subcellular learning mechanisms.

Electrical recordings *in vivo* reveal associations between patterned neural activity in various brain regions and behavior. In hippocampus and striatum, activity at theta frequencies (5-11 Hz) is modulated with learning (Tort et al., 2008). For instance in the dorsal striatum of behaving animals, neuron firing aligns more strongly to a theta rhythm during salient task points such as initiation, completion, and decision-making moments (Tort et al., 2008;DeCoteau et al., 2007). Theta-burst stimulation, which mimics a normal pattern of hippocampal activity, induces a robust LTP in hippocampal area CA1 (Larson and Lynch, 1986;Abraham and Huggett, 1997). The ability of theta-burst stimulation to induced LTP in the hippocampus together with the emergence of theta-rhythms in dorsal

striatum during learning suggests that similar protocols may induce physiologically realistic LTP in *ex vivo* striatum. In this study we test the hypothesis that learning-related temporal patterns, in the form of theta-burst stimulation (TBS), will induce long lasting striatal LTP. We find that delivering stimuli in physiological bursts at a behaviorally relevant theta-range frequency induces striatal LTP in adult tissue without altering ionic composition, and we identify molecular effectors serving striatal theta-burst LTP.

Materials and Methods

All animal handling and procedures were in accordance with the National Institutes of Health animal welfare guidelines and were approved by the George Mason University IACUC. Male C57BL/6 mice (2-5 months) were decapitated while anesthetized using isoflurane. Brains were extracted into ice-cold, oxygenated slicing solution (in mM: KCL 2.8, Dextrose 10, $NaHCO_3$ 26.2, NaH_2PO_4 1.25, $CaCl_2$ 0.5, Mg_2SO_4 7, Sucrose 210) and coronally sectioned at 350µm using a vibratome (Leica VT 1000S). Slices were collected anterior to and including the level of the anterior commissure. Slices were bisected by hemisphere and placed in an incubation chamber containing artificial cerebrospinal fluid (aCSF; in mM: NaCl 126, NaH_2PO_4 1.25, KCl 2.8,$CaCl_2$ 2, Mg_2SO_4 1, $NaHCO_3$ 26.2, Dextrose 11) at 33°C for 30 minutes, then removed to room temperature (21-24°C) for at least 90 minutes before use. All experiments used aCSF containing 1mM Mg^{2+}.

Hemislice pairs were transferred to a submersion recording chamber (Warner Instruments) perfused with oxygenated aCSF (30-32°C) containing 50µM picrotoxin at 2.5-3 mL/min. Pipettes were pulled from borosilicate glass (Sutter P-2000) and filled

with aCSF (resistance 3-6 MΩ). Raw data was recorded using an intracellular electrometer (IE-251A, Warner Instruments) and 4-pole Bessel filter (Warner Instruments), sampled at 20 kHz and processed using a PCI-6251 and LabView (National Instruments). Population spikes were evoked by stimulating white matter overlaying striatum with a tungsten bipolar electrode at an intensity producing 40-60% of the peak signal amplitude on an input-output curve. In most recordings, the synaptically-evoked striatal population spike (N2) was preceded by a downward voltage deflection (N1) indicating afferent depolarization by applied current (Malenka and Kocsis, 1988;Takagi and Yamamoto, 1978). Experiments in which N1 varied by more than 20% from baseline at any point in an experiment were excluded, and post-hoc analysis of the optimal TBS group (50 Hz burst and 10.5 theta) shows no correlation between normalized N1 and N2 values 120 minutes post induction (R-squared= 0.058); thus for analyzed experiments, change in population spike amplitude is not attributable to change in N1. Population spikes were sampled at 0.033 Hz before and after induction. Population spike amplitude was extracted automatically from raw data using the software IGOR. 40ms of raw data is saved surrounding each test-pulse, within which the most negative voltage following the stimulation artifact is subtracted from the more positive of the following two features: either (a) mean voltage averaged over one millisecond immediately preceding the stimulation artifact, or else (b) the upward going peak dividing N1 and N2, as described in (Lovinger et al., 1993a). The absolute value of this difference defines the population spike amplitude. During automated amplitude extraction, traces from each experiment were graphically displayed to be reviewed by eye, guarding against errors in data

extraction. Significant increase or decrease in population spike amplitude relative to average baseline amplitude indicates LTP or LTD, respectively.

All induction paradigms were matched in delivering a total of 400 stimuli (Figure 1A). Theta-burst stimulation (TBS) consisted of ten trains, each delivering ten bursts of four stimuli. The "intra-burst" parameter defined the frequency of stimuli within bursts and was set to either 50 Hz or 100 Hz. The "theta" parameter defined the frequency of bursts within trains and was set to 5 Hz, 8 Hz, or 10.5 Hz. Non-bursty stimulation consisted of ten trains, each delivering 40 stimuli at 50 Hz. The inter-train interval for TBS and non-bursty stimulation was 15 seconds; thus the induction period lasted ~2.5 minutes for both TBS and non-bursty stimulation. High frequency stimulation (HFS) consisted of four trains of 100 stimuli delivered at 100 Hz. Moderate frequency stimulation (20 Hz) consisted of four trains of 100 stimuli delivered at 20 Hz. The inter-train interval for HFS and 20 Hz was 10 seconds; thus the HFS induction required 34 seconds and the 20 Hz induction required 50 seconds.

In pharmacology experiments, TBS was delivered to one hemislice and the other served as a non-stimulated control for non-specific drug effects on signal size. Drugs were bath applied at least 20 min prior to induction, and maintained throughout experiments. Salts were purchased from Fisher Scientific, picrotoxin, chelerythrine chloride, PKI 14-22 amide, telenzepine dihydrochloride, and AIDA were purchased from Tocris, and both APV and SCH23390 were purchased from Enzo. All drugs stocks were dissolved in water.

Raw data analysis and figures were made in IGOR and statistical analysis utilized SAS (v9.2) using the procedure GLM (general linear models); post-hoc tests used LSmeans with Bonferroni adjustment for multiple comparisons. Statistical analysis was performed on the population spike amplitude averaged over a 10 minute interval surrounding every 30 minute increment recorded post-induction, and normalized to the pre-stimulated baseline. Percent change from baseline is reported ± SEM. For graphs and statistical analysis, n is number of experiments, with not more than one experiment per slice, and not more than two identical treatments collected from the same animal.

Results

Corticostriatal LTP is improved by approximating physiological frequencies

Using field recordings in the dorsal striatum, we examined the efficacy of TBS paradigms to induce corticostriatal LTP in dorsomedial (DM) and dorsolateral (DL) striatum in adult mouse brain slice bathed in aCSF containing physiological Mg^{2+} (1mM). $GABA_A$ activity was consistently blocked to isolate the striatal response to glutamatergic synapses. Distinct protocols to be compared were administered to neighboring coronal hemislices in a common chamber. Two temporal features of the induction pattern were varied: intra-burst frequency and theta-burst frequency (Figure 1A).

Intra-burst frequencies of 50 and 100 Hz were compared while maintaining 10.5 Hz theta frequency. Our results indicate that 50 Hz intra-burst produces stronger LTP than 100 Hz in both dorsomedial and dorsolateral regions (Figure 1B). Both 50 and 100 Hz produced a significant LTP compared to non-stimulated controls. Statistical analysis

of the results (Table 1) using repeated measures GLM shows that 50 Hz LTP was significantly better than 100 Hz, with 50 Hz producing larger and longer lasting LTP than 100 Hz, and dorsomedial striatum supporting stronger potentiation than dorsolateral (at 60 minutes: intra-burst $F(2,57)=11.55$, $p<0.0001$; region $F(1,57)=10.07$, $p=0.003$). The more pronounced 50 Hz LTP was recorded out to 120 minutes (see Figure 1C), by which time dorsomedial striatum was potentiated 129±5% and differed significantly from non-stimulated controls (93 %) while dorsolateral striatum, at 106±4%, did not (data not shown) (at 120 minutes: GLM on intra-burst $F(2,37)=21.37$, $p<0.0001$; LSmeans vs. non-stimulated controls DM $p<0.0001$, DL $p=0.1$). Since dorsomedial LTP was stronger than dorsolateral LTP, we subsequently focused on the dorsomedial striatum, using the more effective 50 Hz intra-burst frequency.

Working in the dorsomedial striatum, we tested the effect of three different frequencies spanning the theta range (5-11Hz): 5 Hz, 8 Hz, and 10.5 Hz. Repeated measures GLM shows significant effects of theta frequency ($F(3,46)=10$, $p<0.0001$ at 120min) and time ($F(3,96)=23.25$, $p<0.0001$) with higher theta frequencies inducing the greatest and longest lasting potentiation (Figure 1C). Post-hoc analysis indicates that the 10.5 Hz group, which produced late-phase LTP by maintaining 129±5% potentiation 120 minutes post-induction, differs significantly from non-stimulated controls (LSmeans vs. controls, $p<0.0001$). The same analysis reveals that LTP evoked by 8 Hz theta is not well maintained, losing significance by 120 min (at 60 min: 128±10%, $p=0.001$; at 120 min: 121±9%, $p=0.05$), and that the small LTP evoked by 5 Hz theta (at 60 min: 116±5%, $p=0.04$) has dissipated by 120 minutes (108±4%, $p=0.13$). These differences in LTP

strength cannot be attributed to different initial amplitude, as average baseline population spike amplitude did not differ among the four DM theta-burst paradigms and non-stimulated controls (GLM, $F(4, 77)=1.49$, $p=0.21$). In summary, the optimal TBS (50 Hz intra-burst; 10.5 Hz theta) is the only protocol that produces a long lasting LTP and thus is used for the remainder of our investigations.

Figure 1

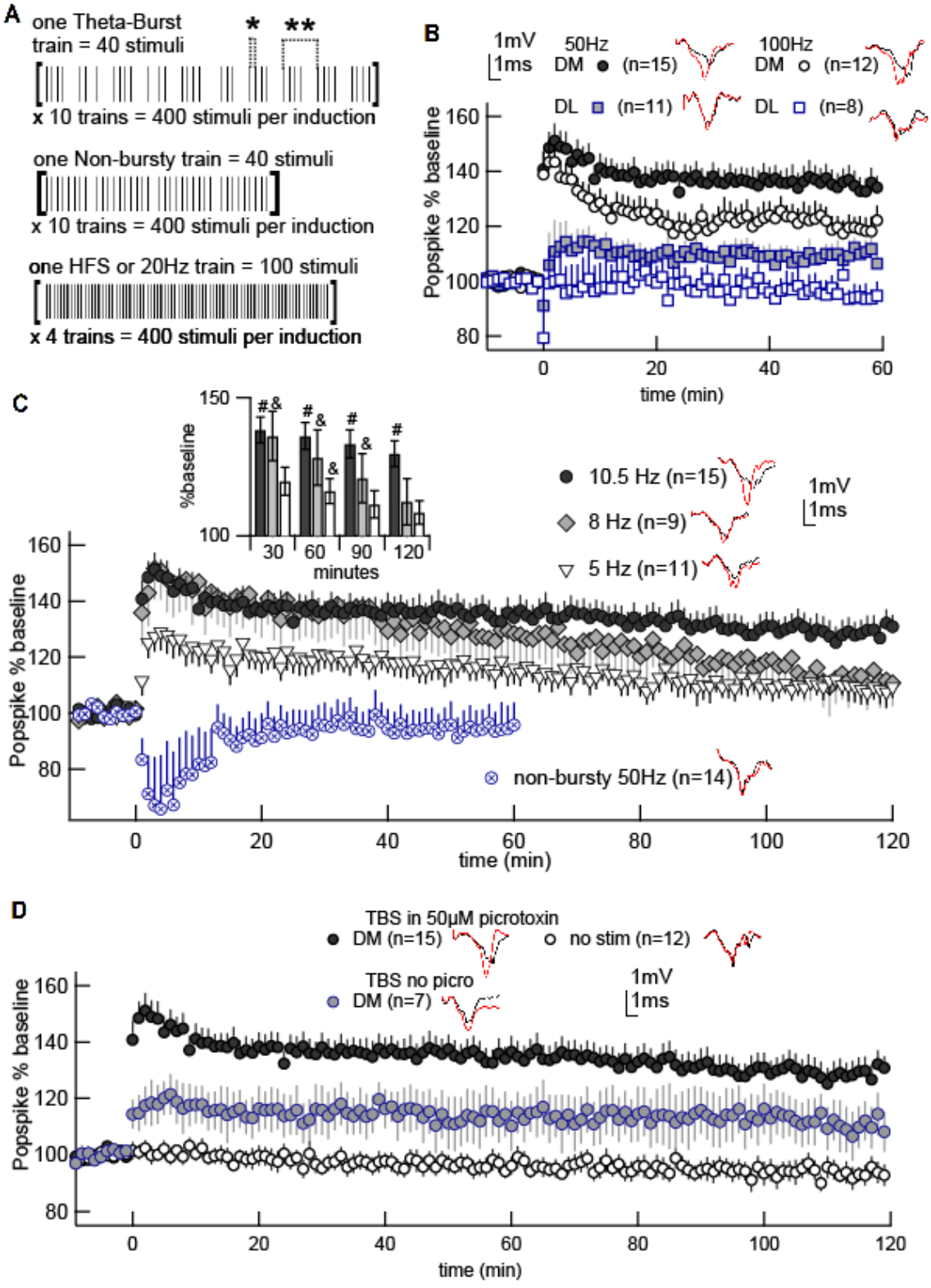
A
one Theta-Burst
train = 40 stimuli
*
**
x 10 trains = 400 stimuli per induction
one Non-bursty train = 40 stimuli
x 10 trains = 400 stimuli per induction
one HFS or 20Hz train = 100 stimuli
x 4 trains = 400 stimuli per induction
B
1mV
1ms
50Hz
DM ● (n=15)
100Hz
DM ○ (n=12)
DL ■ (n=11)
DL □ (n=8)
Popspike % baseline
time (min)
C
%baseline
minutes
10.5 Hz (n=15)
8 Hz (n=9)
5 Hz (n=11)
non-bursty 50Hz (n=14)
1mV
1ms
Popspike % baseline
time (min)
D
TBS in 50µM picrotoxin
DM (n=15)
no stim (n=12)
TBS no picro
DM (n=7)
1mV
1ms
Popspike % baseline
time (min)

Figure 1. LTP depends on intra-burst and theta-burst timing. Example traces from end of experiment (red) overlay traces from baseline (grey) in the insets. Error bars represent ±SEM. **A.** Schematic of induction variants. For each induction paradigm employed in this paper, a single train of stimuli is illustrated in brackets, and annotated to show stimuli number is matched across conditions. Theta-burst (*intra-burst period, **theta period) and non-bursty trains (50 Hz) are delivered with a 15 second inter-train interval. HFS (100 Hz) and 20 Hz trains are delivered with a 10 second inter-train interval. **B.** Intra-burst frequency of 50 Hz is more effective than 100 Hz, both dorsomedial (DM) and dorsolateral (DL). Theta-burst frequency is 10.5 Hz for all groups. **C.** Burst timing is critical to LTP. Theta-burst frequency of 10.5 Hz produces stronger, longer-lasting potentiation than 5 or 8 Hz in DM striatum. Bar graph indicates difference from non-stimulated controls at significance of $p<0.0001$ (#) or $p<0.05$ (&). In the non-bursty condition, the 40 stimuli within each train are delivered at a constant 50 Hz, and neither LTP nor LTD results. Non-bursty experiments ended at 60 min since long-term plasticity was not induced. **D.** Picrotoxin decreases but does not eliminate induction of LTP using the optimal theta-burst timing of 50 Hz intra-burst and 10.5 Hz inter-burst.

Burstiness is critical to striatal TBS LTP

We find that lower intra-burst and higher theta frequencies are more effective for LTP induction; however, as theta frequency increases, the pause separating bursts is reduced. We therefore tested the importance of burst-patterning by eliminating the theta component of our induction protocol by decreasing the inter-burst pause from 35 ms (using the optimal 10.5 Hz theta) to 20 milliseconds. In other words, we delivered trains of stimuli at an unbroken 50 Hz in a "non-bursty" induction variant in which train number, inter-train interval, and the number of stimuli delivered remained matched to TBS protocols (Figure 1A). Despite close temporal similarity to the optimal TBS, the non-bursty stimulation failed to evoke LTP (Figure 1C, Table 1). Statistical analysis implicates burstiness as a significant factor contributing to LTP induction (repeated measures GLM, $F(2,34)=13.89$, $p<0.0001$). Post-hoc analysis indicates significant

difference between TBS and non-bursty groups (LSmeans, $p<0.05$) and no difference between non-bursty stimulation and non-stimulated controls (LSmeans, $p>0.05$). The 35 ms pause between bursts when using the optimal 10.5 Hz theta frequency provides a mere 15 millisecond increase relative to the 20 millisecond break dividing 50 Hz stimuli within non-bursty trains. Our data identify this brief pause as a critical feature enabling long lasting TBS LTP.

TBS LTP is present, though less consistent, when $GABA_A$ inputs remain active

To isolate the contribution of glutamatergic synapses onto medium spiny neurons, TBS-optimization was carried out in 50μM picrotoxin, eliminating GABAergic interneuron and medium spiny collateral influence. Thus, to assess the effect of GABAergic inputs on TBS induced synaptic plasticity, the optimal TBS was administered to the dorsomedial striatum as before, but picrotoxin was omitted from the ACSF. In the absence of picrotoxin, the net effect of TBS remains LTP (Figure 1D, Table 1). On average, population response following TBS in the absence of picrotoxin remained larger than non-stimulated controls (GLM, $F(1,18)=4.59$, TBS without picrotoxin at 60 min: 114±9% vs. controls 95±3%, $p=0.02$; TBS without picrotoxin at 120 min: 110±7% vs. controls 93±4%, $p=0.01$). However, isolation of glutamatergic influence on medium spiny neurons using picrotoxin improves consistency in TBS-evoked LTP; therefore, picrotoxin was used in all subsequent investigations.

Bidirectional plasticity is obtained through temporal pattern

We tested the ability of our preparation to express bidirectional plasticity in order to validate the utility of our theta-burst paradigm for evaluating how temporal pattern

influences plasticity. First, we applied high frequency stimulation (HFS, see Figure 1A) commonly used to induce corticostriatal LTD in the presence of Mg^{2+} (Lerner and Kreitzer, 2011), though in some instances it evokes LTP (Fino et al., 2005) or variable plasticity (Akopian et al., 2000;Akopian and Walsh, 2006;Spencer and Murphy, 2000). Applying the HFS protocol we induced a small, transient increase in signal size dorsomedially (Figure 2, Table 1) and induced no plasticity dorsolaterally (Figure 2, Table 1). A variant of this protocol in which stimulation intensity during HFS is increased produced a similar result (data not shown). In summary, HFS did not produce a significant difference from non-stimulated controls at 30 minutes (GLM, $F(1,29)=0.01$, $p=0.93$).

Next we evaluated a more moderate frequency induction paradigm as this has shown success in promoting striatal LTD (Ronesi and Lovinger, 2004;Lerner and Kreitzer, 2012). In both striatal regions we delivered pulses in the same four-train structure as HFS, but employed a moderate 20 Hz frequency within trains (see Figure 1A). Four trains of 20 Hz evoked LTD, with dorsolateral striatum showing greater LTD than dorsomedial striatum (Figure 2; repeated measures GLM, $F(2,49)=16.46$, region $p=0.03$, stimulation $p<0.0001$). The ability of the 20 Hz stimulation to evoke LTD dorsomedially demonstrates the capacity of our adult tissue preparation to reliably display LTD as well as LTP through manipulation of temporal pattern alone.

Figure 2

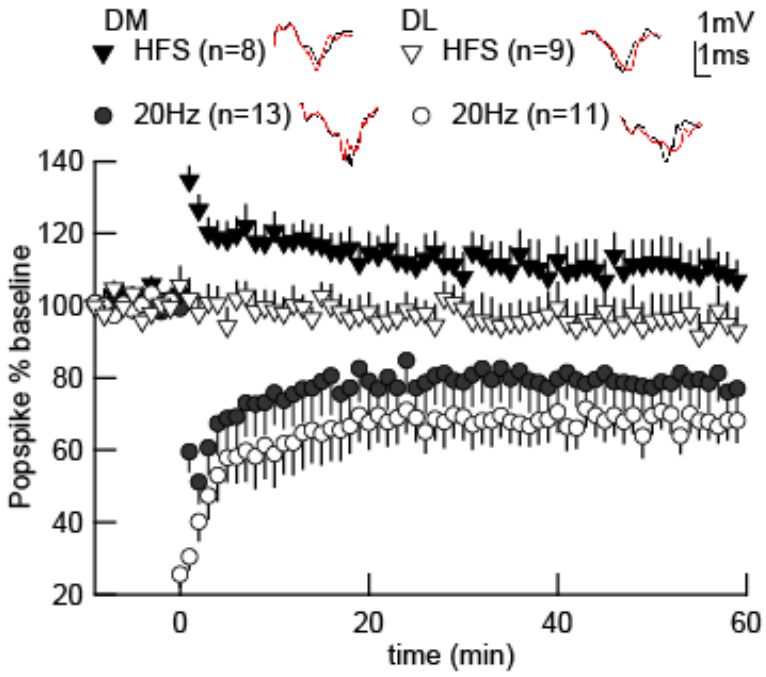

Figure 2. LTD confirms bidirectional plasticity in adult dorsal striatal slice. Four-trains of moderate frequency stimulation (20 Hz), but not high frequency stimulation (HFS: 100 Hz), evokes LTD both DM and DL. Example traces from end of experiment (red) overlay baseline traces (grey). Error bars represent ±SEM.

#Non-stimulated controls . These did not differ regionally , thus DM and DL controls were pooled for analysis.
**Indicates no picrotoxin was used.
50μM picrotoxin is present under all other conditions.
*Indicates the non -bursty induction variant.

Table 1: Plasticity by region and induction variant

region	Intra - burst (Hz)	Theta (Hz)	4train (Hz)	% change 60 min	% change 120 min
DM/L#	-	-	-	95±3	93±4
DM	50	10.5	-	135±5	129±5
DM	100	10.5	-	119±4	-
DL	50	10.5	-	110±4	106±4
DL	100	10.5	-	95±4	-
DM	50	8	-	128±10	121±9
DM	50	5	-	116±5	108±4
DM	50 **	10.5**	-	114±9	110±7
DM	50 *	12.5 *	-	97±7	-
DM	-	-	100	109±6	-
DM	-	-	20	80±7	-
DL	-	-	100	96±6	-
DL	-	-	20	68±5	-

Theta-burst LTP requires NMDA, G_q- and $G_{s/olf}$-coupled receptors

Collaborative signaling by neurotransmitters glutamate, acetylcholine and dopamine is critical to striatal learning and plasticity (Lerner and Kreitzer, 2011). Glutamate at active NMDA receptors provides calcium influx supporting learning and LTP. Metabotropic glutamate receptors on medium spiny neurons have demonstrated involvement in LTP using 0 Mg^{2+} HFS (Gubellini et al., 2003). Theta-burst may optimize acetylcholine release (Zhang et al., 2010), potentially activating G_q coupled signaling pathways in common with mGluR (Calabresi et al., 1999;Calabresi et al., 1998).

Dopamine acting at $G_{s/olf}$-coupled D1-type (D1 and D5) dopamine receptors is critical to LTP in both populations of medium spiny neurons (Pawlak and Kerr, 2008;Kerr and Wickens, 2001). We bath applied antagonists specific to these receptors in order to evaluate their role in TBS LTP. Simultaneous recordings from paired hemislices, one non-stimulated and one TBS-stimulated, controlled for non-specific drug effects. A contemporaneously interleaved cohort of drug-free TBS (50 Hz intra-burst, 10.5 Hz theta) is used for comparison.

Figure 3 illustrates the effects of receptor antagonists on TBS-induced plasticity. The NMDA-type glutamate receptor antagonist APV (50μM) fully prevents TBS LTP (Figure 3A, Table2; GLM, $F(2,33)=48.85$, $p<0.0001$), confirming a requirement for NMDA receptor activation. Next, we independently block m1 type metabotropic acetylcholine (m1 AChR) and group I glutamate (mGluR1/5) receptors. Both the m1 AChR antagonist AIDA (100μM) and mGluR1/5 antagonist telenzepine (300nM) individually abolish TBS LTP without affecting unstimulated control slices (Figure 3B-C, Table2; AIDA: GLM, $F(2,41)=14.04$, $p<0.0001$; telenzepine: GLM, $F(2,35)=28.93$, $p<0.0001$). This suggests that G_q activation is needed both through glutamate and acetylcholine, as neither is sufficient to support TBS LTP alone. Bath application of an antagonist selective for $G_{s/olf}$-coupled dopamine receptors, SCH23390 (10μM), abolishes TBS LTP by 30 minutes without affecting unstimulated control slices (Figure 3D, Table 2; SCH23390: GLM, $F(2,36)=26.02$, $p<0.0001$), confirming a requirement for dopamine activation of $G_{s/olf}$-coupled pathways. Post-hoc analysis for each of the above antagonists shows no difference in population spike amplitude over time between non-stimulated and

TBS-treated slices in the presence of drug (LSmeans, p>0.9). These results confirm that TBS LTP shares receptor dependence with striatal learning and established plasticity.

Figure 3

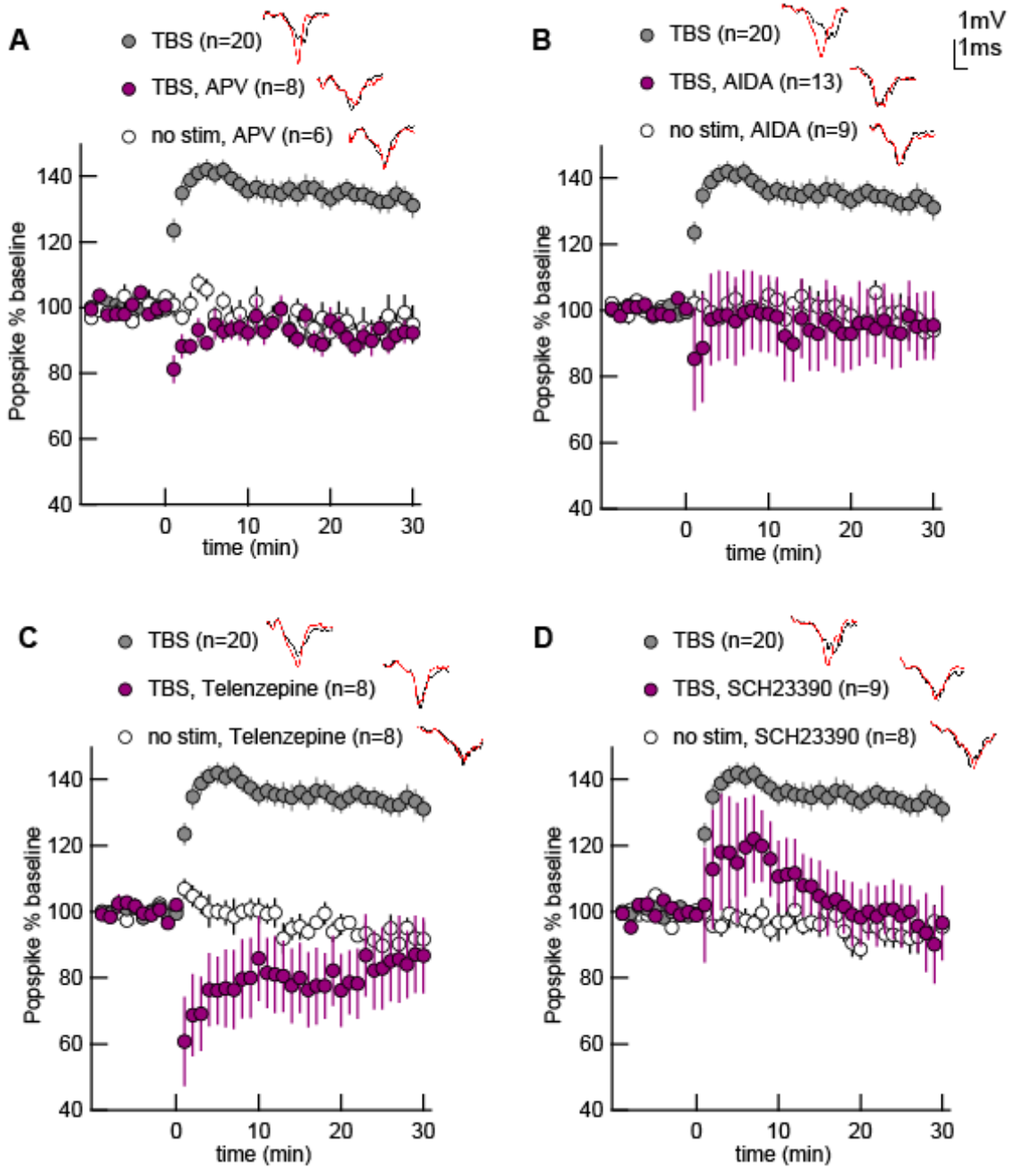

Figure 3. TBS LTP requires NMDA , type I mGluR, m1 AChR, and dopamine D1-type receptors. The drug-free TBS group (DM, 50 Hz intra-burst, 10.5 Hz theta) was collected interleaved with pharmacology experiments, and thus is different than the analogous TBS group in Fig 2. Example traces from end of experiment (red) overlay baseline traces (grey). Error bars represent ±SEM. **A.** NMDA receptor antagonist APV blocks LTP. **B.** type I mGluR antagonist AIDA blocks LTP. **C.** m1 AChR antagonist telenzepine blocks LTP. **D.** D1-type receptor antagonist SCH23390 blocks LTP.

Theta-burst LTP requires PKC, PKA and ERK

Next we tested several kinases downstream of these implicated receptors to identify further effectors serving TBS LTP. The combination of NMDA-derived calcium and G_q-signaling creates the potential for activating protein kinase C (PKC), a kinase which may serve striatal LTP (Gubellini et al., 2004;Calabresi et al., 1998). $G_{s/olf}$-signaling elevates cAMP and activates protein kinase A (PKA), a second kinase with a likely role in striatal LTP (Spencer and Murphy, 2002). Extracellular signal-regulated kinase (ERK) is a kinase activated downstream of either PKC or PKA and has important roles in memory, drug addiction and long lasting plasticity (Mazzucchelli et al., 2002;Shiflett and Balleine, 2011). We bath applied antagonists to these kinases during TBS experiments, again recording from paired hemislices, one non-stimulated and one TBS-stimulated, to control for non-specific drug effects.

Figure 4 illustrates the effects of kinase antagonists on TBS-induced plasticity. Bath applied PKC antagonist, chelerythrine (6-10μM), significantly reduces TBS LTP without affecting unstimulated control slices (Figure 4A, Table 2; GLM, $F(2,33)=37.27$, $p<0.0001$). Similarly, bath applied cell-permeant PKA inhibitor peptide, PKI (1μM), significantly reduces TBS LTP without affecting unstimulated control slices (Figure 4B,

Table 2; GLM, $F(2,39)=25.28$, $p<0.0001$). Since PKI did not completely block LTP, we evaluated its effect in combination with chelerythrine. We used reduced-concentrations of both antagonists, each showing reduced efficacy to block LTP (Figure 4; reduced CHE at 30 min: 114±10; reduced PKI at 30 min: 117±10). This reduced-concentration combination fully prevents TBS LTP (Figure 4C, Table 2; GLM, $F(2,33)=15.31$, $p<0.0001$), demonstrating that PKC and PKA cooperatively support striatal LTP. Bath applied MAPK/ERK kinase (MEK) inhibitor U0126 (30μM) prevents MEK from activating ERK, and fully blocks TBS LTP (Figure 4D, Table 2; GLM, $F(2,32)=18.4$, $p<0.0001$); this effect is similar to the combination of antagonists to PKA and PKC, either of which can act upstream of ERK. Our results newly implicate PKC in activity-dependent striatal LTP, and agree with prior studies implicating PKA and ERK (Calabresi et al., 1992b;Kerr and Wickens, 2001). These results further suggest that PKC and PKA cooperatively serve LTP, which could occur through co-activation of ERK.

Figure 4

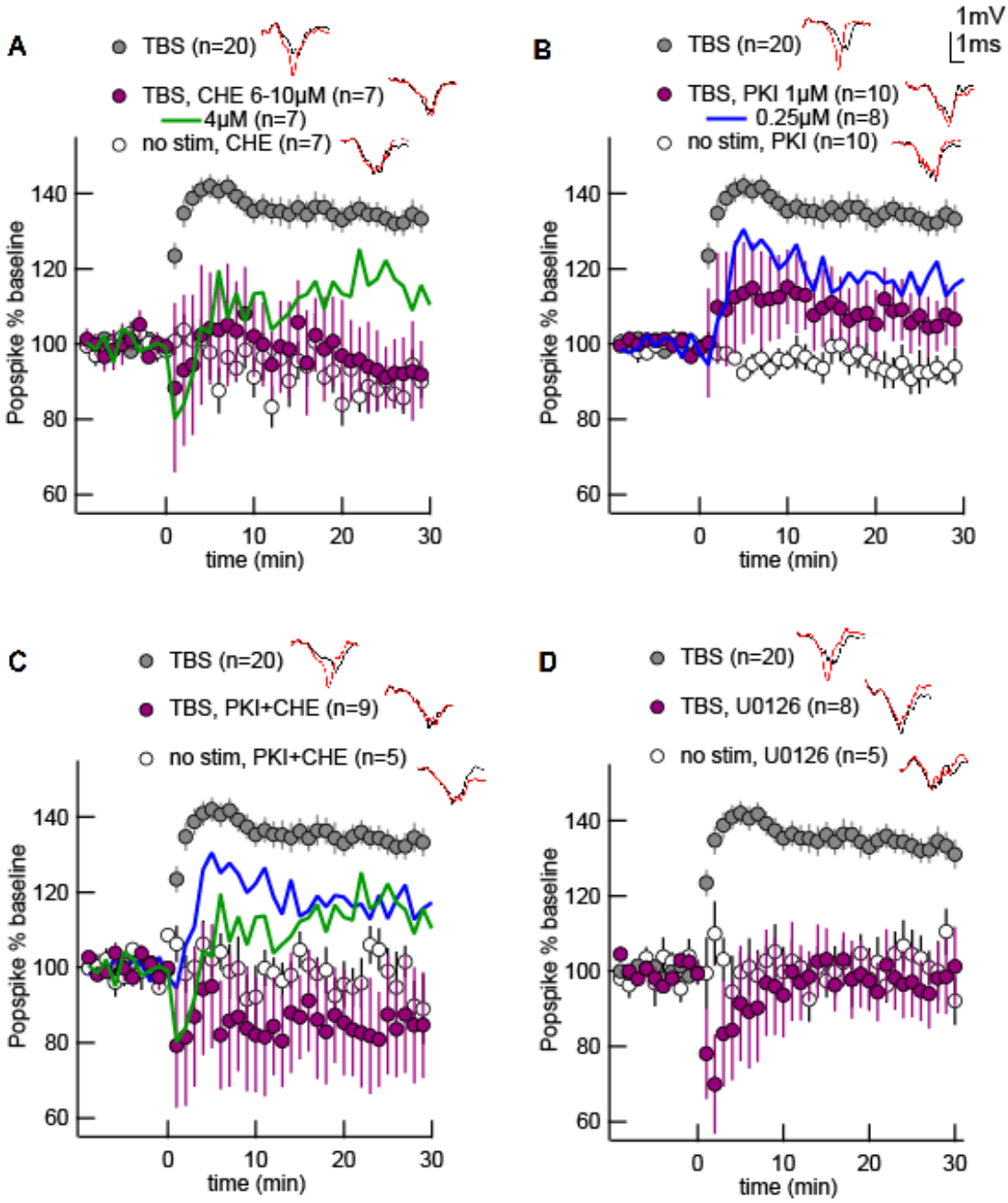

Figure 4. TBS LTP requires PKC, PKA and ERK. The drug-free TBS group (DM, 50 Hz intra-burst, 10.5 Hz theta) was collected interleaved with pharmacology experiments, and thus is different than the analogous TBS group in Fig 2. Example traces from end of experiment (red) overlay baseline traces (grey). Error bars represent ±SEM. **A.** PKC

inhibitor chelerythrine blocks LTP. Reduced drug concentration reduces amplitude without completely blocking LTP (green). **B.** PKA inhibitor PKI blocks LTP. Reduced drug concentration reduces amplitude without completely blocking LTP (blue).
C. Reduced concentrations of PKC and PKA inhibitors fully block LTP when combined. Mean effect from independent reduced concentration inhibitors are overlaid.
D. Preventing ERK activation with MEK inhibitor U0126 blocks LTP.

Table 2: Pharmacology indicating LTP effectors

drug name	inhibits	% change 30 min, TBS	% change 30 min, no stim
APV	NMDA receptor	91±2	95±4
AIDA	type I mGluR	95±10	96.3 ±6
telenzepine	m1 receptor	85±12	92±4
SCH 23990	D1/5 receptor	97±6	94±3
CHE	PKC	92±10	89±4
PKI	PKA	105±8	92±4
CHE+PKI	PKC & PKA	86±15	93±7
U0126	ERK	97±9	101±6

Discussion

Learning correlates with theta frequency neural activity in the dorsal striatum, suggesting a theta-burst induction paradigm might evoke behaviorally relevant LTP in striatum. Our results support this hypothesis by showing that TBS evokes LTP which is more pronounced when using temporal parameters with better correspondence to striatal physiology. We further determine that a critical induction feature for LTP is burstiness, which is intriguing since medium spiny neuron up-state potentials observed in organotypic culture and *in vivo* may facilitate the burst firing that has demonstrated

importance for striatal network function and behavior (Stern et al., 1997;Kerr and Plenz, 2002;Miller et al., 2008). Importantly, reliance on *in vivo* striatal theta rhythms rather than altered ionic composition or pharmacology makes TBS LTP a convincing *ex vivo* model for plasticity serving learning, memory and motor adaptation. Indeed, we confirm involvement of several receptors and kinases previously implicated in striatal plasticity as well as learning and memory. The success of TBS LTP across dorsal striatal regions in adult brain slice presages its utility in combination with future behavioral studies.

Though both 50 Hz and 100 Hz stimulation frequencies have been applied to evoke plasticity, the striatal medium spiny neurons comprising 95% of striatal cells are not likely to be engaged by high frequency activation *in vivo*. These neurons receive input from layer V cortical neurons which fire with an average rate of 5-10 Hz (Fellous et al., 2003;Wilson and Groves, 1981). Furthermore, recordings from behaving mice and rats have shown medium spiny neurons fire below 5 Hz on average, with the maximum spontaneous firing rate *in vivo* no greater than 50 Hz (Barnes et al., 2005;Miller et al., 2008). In anesthetized rat, single striatal neurons are successfully entrained to moderate (20 Hz) but not to high frequency (100 Hz) frequency cortical afferent stimulation (Schulz et al., 2011). Given these observations in the literature, we expected and indeed obtained the greatest plasticity through use of more moderate induction frequencies.

Theta frequency is a physiologically significant parameter in activity-based LTP induction as dorsal striatal local field potentials recorded *in vivo* demonstrate neuronal population coherence at theta-range frequencies (5-11 Hz). Importantly, these theta rhythms are modulated in an activity-dependent manner during learning (Buzsaki,

2005;Koralek et al., 2012;Tort et al., 2008). Depolarizing potentials in medium spiny neurons occur at 5 Hz as a result of 5 Hz coherence in firing among hundreds of convergent afferents from layer V cortex in anesthetized rat (Charpier et al., 1999). Higher theta-range frequencies may dominate in wakeful animals, or during learning, since recent studies in awake, behaving subjects indicate that learning-related theta centers around 7-11 Hz in dorsal striatum (DeCoteau et al., 2007;Tort et al., 2008). Nonetheless, we initially used 5 Hz TBS because TBS evokes robust LTP in hippocampal slice (Larson et al., 1986;Nie et al., 2007), and striatal STDP pairings paced at 5 Hz evoke LTP in young animals (Shen et al., 2008). While 5 Hz indeed evoked a modest LTP, we found the amplitude and duration was greatly improved by using higher frequency theta-bursts. This result suggests that LTP processes may be tuned to subtly different frequencies in striatum versus hippocampus. In light of the recent *in vivo* work mentioned, this result supports the idea that striatal neurons are tuned to promote LTP in response to temporal patterns emerging with learning behavior.

In optimizing a theta-burst protocol, we eliminated fast actions of intra-striatal GABA release which are present *in vivo* in order to provide certainty that TBS potentiates the response of medium spiny neurons to glutamatergic afferents rather than depressing the fast GABAergic inhibition of striatal response. This is a valid concern because GABAergic synapses within striatum are more sensitive to endocannabinoid-dependent depression than are glutamatergic synapses (Adermark and Lovinger, 2009). Though most corticostriatal plasticity studies are carried out with $GABA_A$ blocked (Kerr and

Wickens, 2001;Shen et al., 2008;Gubellini et al., 2003;Akopian and Walsh, 2006), native $GABA_A$ transmission must shape striatal plasticity.

Indeed, the direction of STDP is reversed by the presence of $GABA_A$ antagonists. Specifically, corticostriatal synapses onto medium spiny neurons are potentiated when pre-synaptic release precedes post-synaptic depolarization (Hebbian LTP) only when $GABA_A$ is blocked; when $GABA_A$ is not blocked pre-post pairing is depressing, and LTP is instead evoked when post-synaptic depolarization preceded pre-synaptic release (anti-Hebbian) (Fino et al., 2010). Several mechanisms have been proposed to account for reversal of STDP by $GABA_A$, such as altered ratio of NMDA to L-type calcium influx in dendrites (Paille et al., 2013) or Hebbian potentiation of feed-forward inhibition (Fino et al., 2008). Alternatively, increased dopamine release may be responsible for switching STDP direction (Shen et al., 2008;Shindou et al., 2011), since $GABA_A$ antagonists increase activity-dependent intra-striatal dopamine release (Juranyi et al., 2003). Any of these mechanisms: altered calcium source, potentiation of feed-forward inhibition, or lowered dopamine release, may contribute to reduce TBS LTP amplitude in the absence of picrotoxin.

The optimal TBS protocol induces robust dorsomedial LTP lasting multiple hours, yet no plasticity results if the brief pause separating bursts is omitted. This demonstrates that the 35 ms pause between bursts is critical to LTP, since no plasticity is induced if this pause is reduced to 20 ms (so that pulses run together into non-bursty, 50 Hz stimuli). Note that TBS variations with lower intra-burst or higher inter-burst frequencies cannot be tested while conserving pulse number per burst, as these

adjustments would encroach on the already small inter-burst pause, eliminating burstiness. The requisite pause may enable LTP through phasic activation of neuromodulators, given that salient behavioral stimuli produce burst firing of cholinergic interneurons (Aosaki et al., 1994) which in turn enhances dopamine release (Threlfell et al., 2012). This may be tested using voltammetry to compare dopamine release resulting from TBS and its non-bursty counterpart. Alternatively, the pause may enable re-sensitization of critical plasticity effectors. For instance, a brief break in stimulation may relieve inactivation of NMDA receptors or else it might relieve desensitization of metabotropic glutamate, dopamine or acetylcholine receptors implicated in this study. Investigating the requisite pause may shed light on a mechanism for the resilience of TBS LTP in the absence of $GABA_A$ antagonist.

Striatal sensitivity to temporal pattern is most meaningful if both LTP and LTD can be evoked in the same preparation; therefore we sought to induce LTD by varying temporal pattern alone. HFS is commonly used to evoke LTD in Mg2+ containing aCSF (Adermark and Lovinger, 2009;Yin et al., 2009;Choi and Lovinger, 1997;Wang et al., 2006), yet 4-train, 100 Hz HFS does not evoke lasting plasticity in our preparation. This may be related to animal age, which is known to influence evoked striatal plasticity (Partridge et al., 2000) and at least one report notes that 100 Hz HFS does not reliably produce LTD in adult animals (Hopf et al., 2010), while other studies report a mixture of LTP and LTD as a result of HFS in adults (Akopian et al., 2000;Akopian and Walsh, 2006;Spencer and Murphy, 2000). Our preparation demonstrates reliable LTD across regions when stimulation is delivered at a moderate, 20 Hz frequency, similar to

protocols used previously (Yin and Lovinger, 2006;Lerner and Kreitzer, 2012). In addition to being more effective, 20 Hz is more physiological than 100 Hz given the moderate native firing frequencies in cortical afferents and striatal medium spiny neurons (Schulz et al., 2011). Capacity for bidirectional plasticity in both dorsomedial and dorsolateral striatum through manipulation of stimuli timing alone argues against our preparation being skewed toward generating LTP. This strengthens our findings that temporal features of TBS, such as frequency-tuning and burstiness, can modulate LTP strength.

In addition to confirming a requirement for NMDA receptors, our experiments demonstrate that TBS LTP requires G_q coupled metabotropic receptors responding to glutamate and acetylcholine, and $G_{s/olf}$ coupled dopamine receptors. G_q effectors interact with calcium influx to generate 2-arachidonyl glycerol, an endocannabinoid implicated in LTD, and also lead to PKC activation. PKC has been implicated in plasticity, memory and in striatal chemical LTP (Diez-Guerra, 2010;Gubellini et al., 2004), and is a critical intermediary for neuromodulation of NMDA and AMPA receptors within striatum (Ahn and Choe, 2010;Calabresi et al., 1998). We find that independently blocking group I mGluR, m1 AChR, or PKC is sufficient to fully prevent TBS LTP, suggesting that coordinated glutamate and acetylcholine transmission is needed to generate LTP-supportive PKC. The neurotransmitter dopamine acts at $G_{s/olf}$ coupled D1-type receptors (D1 and D5) expressed on several cell classes within striatum, including both classes of medium spiny neuron (Rivera et al., 2002;Surmeier et al., 1996). D1-type dopamine receptors (along with A2A adenosine receptors) are $G_{s/olf}$-coupled, leading to elevations

in cyclic AMP and PKA. PKA has demonstrated a role in learning, and is believed to serve striatal LTP by enhancing medium spiny neuron responsiveness (Tseng et al., 2007;Dudman et al., 2003). Our finding that D1-type dopamine receptor antagonist blocks LTP is consistent with studies of striatal LTP induced using either 4-train HFS in zero-Mg^{2+} or STDP (Kerr and Wickens, 2001;Calabresi et al., 1992a;Shen et al., 2008;Pawlak and Kerr, 2008;Fino et al., 2010). Indeed, D1-type receptor antagonism blocks 0 Mg^{2+} LTP equally well in all patched medium spiny neurons, with the same time course we show (Kerr and Wickens, 2001;Calabresi et al., 2000b). Thus, D1/D5 receptor activity likely increases PKA within medium spiny neurons, which we demonstrate contributes to LTP. Activation of PKA has been demonstrated to accelerate degradation of G_q proteins needed to generate endocannabinoids and active PKC (Lerner and Kreitzer, 2012); thus the requirement for two sources of G_q may stem from the need to overcome PKA obstructing PKC activation. Together our results demonstrate that neither G_q nor $G_{s/olf}$ signaling is independently sufficient to support TBS LTP, and that both contribute.

Persistent memory, late-phase plasticity and long lasting TBS LTP are each reliant on the kinase and transcriptional regulator ERK (Adams et al., 2000;Adams et al., 2000;Valjent et al., 2001). ERK is important for striatal learning, especially that associated with drug addiction (Shiflett and Balleine, 2011;Valjent et al., 2006). It is noteworthy that both kinases PKC and PKA are capable of raising ERK phosphorylation and activity (Mao et al., 2005;Shiflett and Balleine, 2011). Thus, the cooperativity we see when combining low concentrations of PKC and PKA antagonists may result from

concomitant reduction in these two sources of ERK phosphorylation. Two possibilities can be distinguished in future works by measuring the effect of PKA and PKC inhibitors on TBS LTP from identified D1 and D2 medium spiny neurons: PKC and PKA may act together upstream of ERK in each medium spiny neuron; or else PKC and PKA may be differentially critical to ERK activation between medium spiny neuron classes. Identifying roles for effectors known to be critical to learning and long term memory storage strengthens TBS as a model for behaviorally relevant plasticity.

We find dorsomedial striatum more prone to potentiation and dorsolateral more prone to depression, agreeing with numerous reports (Wickens et al., 2007;Smith et al., 2001;Partridge et al., 2000). Striatal regional gradients exist for several plasticity effectors. For instance, LTD-required endocannabinoid receptors are denser laterally (Hilario et al., 2007). An established medial to lateral gradient in NMDA receptor subunit composition and distribution (Yin et al., 2009;Chapman et al., 2003) may cause regional differences in calcium-dependent plasticity effectors including PKC. Dorsolaterally, greater dopamine innervation paired with higher density $G_{i/o}$ coupled D2-type dopamine receptors (Yin et al., 2009;Doucet et al., 1986) could limit LTP-supportive PKA in this region. The trend toward greater LTP in dorsomedial relative to dorsolateral striatum is maintained whether or not $GABA_A$ is blocked (Smith et al., 2001); and our use of picrotoxin rules out regional differences in plasticity of $GABA_A$ transmission. Whatever the reasons for greater LTP magnitude and duration dorsomedially, the current utility of TBS LTP in either region (albeit reduced laterally) will be valuable for investigating

learning since dorsal striatal regions serve distinct styles and phases of learning (Yin et al., 2006;Pauli et al., 2012).

Importantly, dorsal striatum expresses long-lasting potentiation in response to physiological activity patterns similar to those occurring with learning, namely theta-burst stimulation. The extensive duration of dorsomedial TBS LTP will accommodate evaluation of late-phase LTP in this region. Utility in adult tissue will benefit behavioral approaches to striatal research since working with adult animals avoids developmental confounds. Moreover, we have success with TBS LTP in tissue from adult Long Evans rat, a more versatile model for behavior than mice (Hawes et al., 2012). Thus TBS improves on existing LTP protocols in its capacity to merge plasticity with behavioral studies, generating exciting opportunity for advancing knowledge of striatal neurobiology serving learning and memory

Grants

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Disclosures

The authors declare no conflict of interest, financial or otherwise.

3. MULTI-MODAL PLASTICITY IN DORSAL STRIATUM WHILE LEARNING A LATERALIZED NAVIGATION TASK

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Abstract

Growing evidence supports a critical role for the dorsal striatum in cognitive as well as motor control. Both lesions and *in vivo* recordings demonstrate a transition in the engaged dorsal striatal subregion - from dorsomedial to dorsolateral - as skill performance shifts from an attentive phase to a more automatic or habitual phase. What are the neural mechanisms supporting the cognitive and behavioral transitions in skill learning? To pursue this question, we utilized T-maze training during which rats

transition from early, attentive (dorsomedial) to late, habitual (dorsolateral) performance. Following early or late training, we performed the first direct comparison of bidirectional plasticity in striatal brain slices, and the first evaluation of striatal plasticity by hemisphere relative to a learned turn. Consequently, we find that long-term potentiation and depression (LTP and LTD) are independently modulated with learning rather than reciprocally linked as previously suggested. Our results establish that modulation of evoked synaptic plasticity with learning depends on striatal subregion, training stage, and hemisphere relative to the learned turn direction. Exclusive to the contralateral hemisphere, intrinsic excitability is enhanced in dorsomedial relative to dorsolateral medium spiny neurons early in training and population responses are dampened late in training. Neuronal reconstructions indicate dendritic remodeling after training, which may represent a novel form of pruning. In conclusion, we describe region- and hemisphere-specific changes in striatal synaptic, intrinsic, and morphological plasticity which correspond to T-maze learning stages, and which may play a role in the cognitive transition between attentive and habitual strategies.

Introduction

Performance of a newly learned task requires more careful attention, separate cognitive processes, and engages different brain regions than skillful performance of the same task after extensive training. Within the basal ganglia, striatal subregions preferentially serve these distinct learning stages (Ragozzino, 2003;Murray et al., 2012). The dorsomedial region is engaged in periods of flexibility in decision-making and serves

early learning, while the dorsolateral striatum streamlines practiced skills and habits later in learning. This shift in striatal engagement is indicated by changes in behavior following subregional lesions (Yin et al., 2004;Lee et al., 2014;Whishaw et al., 1987), *in vivo* neural activity (Thorn and Graybiel, 2014;Yin et al., 2009), and changes in glutamate receptor contribution or composition suggestive of synaptic plasticity (Kent et al., 2013;Yin et al., 2009;Shan et al., 2014).

Synaptic plasticity is the activity dependent adjustment in connections between neurons; within striatum, this enables experience to selectively enhance critical action-outcome associations. The only study to date demonstrating learning-related change in evoked plasticity across striatal subregions reports enhanced long-term depression (LTD) and altered AMPA:NMDA ratios in the dorsolateral striatum of extensively-trained animals (Yin et al., 2009). One interpretation is that recent long-term potentiation (LTP) elevates synaptic weight, thereby enhancing room for synaptic weight change in the opposing direction, i.e. LTD (Cooper and Bear, 2012;Lin, 2010). Alternatively, learning may modulate LTD and LTP independently rather than reciprocally. Distinguishing these possibilities requires direct comparison of bidirectional plasticity, which we achieve using a novel theta-burst LTP protocol (Hawes et al., 2013).

T-maze training transitions rats from action-outcome to stimulus-response performance of a rewarded turn (Packard, 1999;Yin and Knowlton, 2004). Hemispheric lesion and *in vivo* recordings demonstrate that turning behavior corresponds to increased striatal activity in the contralateral hemisphere (Ungerstedt et al., 1969;Cui et al., 2013). However the development of plasticity sculpting a learned turn is uncharacterized,

making a lateralized task useful. The present study tracks plasticity by hemisphere during T-maze learning in order to identify the hemispheric distribution of plasticity sculpting a turn.

Neuronal excitability and morphology may interact with synaptic plasticity to serve learning. In dorsal striatum, potassium channel regulation accompanies spatial learning (Truchet et al., 2012), and modifies plasticity (Nazzaro et al., 2012). Dendritic spine growth is cited as an indication of LTP (Kasai et al., 2010), while a recent work shows that memory and LTP are supported by spine loss in behaviorally-engaged circuits, suggesting signal to noise enhancement through synaptic pruning (Sanders et al., 2012). To directly evaluate whether these different forms of plasticity interact to produce learning behavior, we measure excitability and morphology of striatal medium spiny neurons in parallel with synaptic plasticity measures.

This is the first study to investigate anatomical distribution of evoked bidirectional striatal plasticity as animals transition from early, attentive *place* to late, automatic *response* strategies with T-maze learning (Packard, 1999). We find learning independently modulates striatal LTD and LTP. Plasticity, excitability, and morphology collaboratively reflect maze learning, and we demonstrate that neural learning signatures have a biased hemispheric distribution reflecting the direction an animal learns to turn.

Materials and Methods

Animals and Habituation

All animal handling and procedures were in accordance with the National Institutes of Health animal welfare guidelines and were approved by the George Mason University IACUC. Adult, male Long-Evans rats (2-3 months old, Charles River Laboratories) were acclimated to the animal facility, undisturbed, for a minimum of one week. After acclimatization, rats were habituated to human handling by passive holding for five minutes a day for seven days, during which time they began food restriction (Fig 1A). To motivate food seeking, rats were maintained between 85% of their initial free-feeding weight and 85% average weight for their age in free-feeding male Long Evans rats (providing for weight-gain with age in late-trained animals). On their seventh day of holding rats were given three Kellogg® Froot-Loop halves in their home cage to begin habituation to this food reward, the same reward used in maze training and probe runs. The next day rats began food cup habituation, in which they explored a rectangular table until eating from a food cup at one end of the table containing one Froot-Loop half. Food cup habituation continued until rats ate from the cup in under 3 minutes on two consecutive days (typically taking 3 days). Holding and food cup habituation occurred in the same room, distinct from housing and maze rooms. Rats were first exposed to the maze room during a single day of maze habituation in which rats were released onto the maze from the opaque South arm start box as would occur during training, and were given five minutes to explore the maze without reward. Including holding, food cup habituation and maze habituation, all rats experienced 11±1.1 days of habituation (Fig

1A). To avoid reinforcing intrinsic bias in turning behavior, experimenters noted the order of arm entry during maze habituation, and rewarded the second-choice arm during maze training. The rewarded turn direction was consistent for each rat, but varied between subjects. Assignment to habituated, early-trained and late-trained groups was pseudo-random and preceded the start of behavior for each rat. Behavior start-dates were staggered such that, on any given day, animals from each condition were in training but final probes would not overlap.

T-maze training

The maze room was dimly-lit to minimize animal anxiety, with bold visual cues distinguishing all quadrants of the room. Maze habituation, training, and probe trials were video recorded by a ceiling-mounted camera centered over the maze. Identical food cups were secured at ends of East and West maze arms, and identical, opaque start boxes were fastened to the ends of South and North arms. A mobile, clear plastic barricade blocked entry into the arm opposite the animal's start position, which was the South arm during training and North arm on probes.

Each training day consisted of four maze runs, and rats were trained every day, except for probe trial days. Rats entered the maze room in an opaque transfer cage and were given approximately 30 seconds in the transfer cage, followed by approximately 10 seconds in the South arm start box before each run. Either the East or else the West arm was baited (Fig 1B left). After each run, rats were removed to the transfer cage after either eating the reward or committing a wrong turn. The maze was wiped down between runs to obscure olfactory cues, and on a pseudo-random schedule, the maze top was

rotated 180 degrees to prevent reliance on intrinsic cues. Criteria to end early-training were correct execution of all four runs within a training day, after a minimum of four days training. On meeting these criteria, a strategy probe was administered the next day (P1, Fig 1A). Late-trained rats were given two additional weeks of training with 6 training days per week, and a single strategy probe every seventh day (P2 and P3, Fig 1A). Early-trained rats trained 5.9±0.4 days (23.6±1.5 runs) while late-trained rats trained 18.6±0.6 days (74.3±2.2 runs) beyond habituation.

On probe days, rats were started in the North arm, both food cups were baited, and rats were given a single run (Fig 1B right). On a probe run, an animal rewarded throughout training for turns toward the East arm was scored as demonstrating a *place* strategy if it made a turn toward the East arm, thereby choosing the spatial location rewarded in training. In contrast the same animal was scored as using a *response* strategy if it made a turn toward the West arm, thereby executing the turn direction rewarded in training. On both probe days and training days, a turn was determined by the entire body and base of the tail crossing into an arm. Vicarious trial and error (VTE) was defined by a nose-cross into an arm followed by nose-cross out of the arm rather than committing to a turn, assessed from the aerial video view.

Slice Preparation

Habituated control rats were sacrificed 24 hours after maze habituation. Trained rats were sacrificed 24 hours following the first probe (early-trained group) or third probe (late-trained). Brain slices were prepared as described in Hawes et al. 2013. Briefly, animals were anesthetized with isoflurane and brains were extracted quickly and placed

in oxygenated ice-cold sucrose slicing solution (in mM: KCL 2.8, Dextrose 10, $NaHCO_3$ 26.2, NaH_2PO_4 1.25, $CaCl_2$ 0.5, Mg_2SO_4 7, Sucrose 210). Coronal slices were cut 350μm thick on a Leica vibrotome (VT1000S), and the animal's right and left hemispheres were carefully tracked and moved to separate, labeled incubation chambers containing aCSF (in mM: NaCl 126, NaH_2PO_4 1.25, KCl 2.8, $CaCl_2$ 2, Mg_2SO_4 1, $NaHCO_3$ 26.2, Dextrose 11) heated to 33°C for 30 minutes and then removed to room temperature (21-23°C) until recording.

Field Recordings

During field recordings, a pair of hemi-slices was transferred to a submersion recording chamber (Warner Instruments) perfused with oxygenated aCSF at 2.5-3 mL/min and 30-32°C containing 50μM picrotoxin (Tocris Bioscience). Pipettes (resistance 3-6 MΩ) were pulled from borosilicate glass on a P-2000 puller (Sutter Instruments) and filled with the same aCSF bathing the tissue. Raw data were recorded using an intracellular electrometer (IE-251A, Warner Instruments) and 4-pole Bessel filter (Warner Instruments), sampled at 20 kHz and processed using a PCI-6251 and LabView (National Instruments). Population spikes were evoked by stimulating white matter overlaying either dorsomedial or dorsolateral striatum with a tungsten bipolar electrode (diameter 0.005'' bare, 0.007'' Teflon-coated, A-M Systems) at an intensity producing 40-60% of the peak signal amplitude on an input-output (IO) curve collected at 0.015 Hz. In most recordings, the synaptically-evoked striatal population spike (N2) was preceded by a downward voltage deflection (N1) indicating afferent depolarization by applied current (Takagi and Yamamoto, 1978;Lovinger et al., 1993b). Experiments in

which N1 varied by more than 20% from baseline at any point in an experiment were excluded. Population spikes were sampled at 0.03 Hz pre- and post-induction. Plasticity induction was accomplished as described in Hawes et al. 2013. Briefly, LTP was induced by theta-burst stimulation (TBS) consisting of ten trains, each train consisting of ten bursts at 10.5 Hz (theta), and each burst consisting of four stimuli at 50 Hz, with trains spaced 15 seconds apart. Using this protocol, LTP was reliably induced only in the dorsomedial striatum of control animals; hence it was not studied dorsolaterally. In both dorsomedial and dorsolateral regions, LTD was induced by moderate frequency stimulation consisting of four trains of 100 stimuli delivered at 20 Hz, with trains spaced 10 seconds apart.

The experimenter was blind to behavioral data during electrophysiology recording and data extraction. Population spike amplitude was extracted automatically from the 40ms of raw data surrounding each test pulse using the software IGOR (Wavemetrics). The most negative voltage (N2) following the stimulation artifact was subtracted from the more positive of the following two features to determine population spike amplitude: either (a) mean voltage averaged over one millisecond immediately preceding the stimulation artifact, or (b) the upward going peak dividing N1 (fiber volley) and N2, as previously described (Hawes et al., 2013;Lovinger et al., 1993b). During automated amplitude extraction, traces from each experiment were graphically displayed for review by eye, guarding against errors in data extraction. Statistical analysis was performed on the population spike amplitude normalized to the pre-induction baseline. Significant

increase or decrease in population spike amplitude relative to average baseline amplitude indicates LTP or LTD, respectively.

Whole-cell Recordings

Single hemi-slices from the same subjects used in plasticity experiments were transferred to a submersion recording chamber (ALA Science) gravity-perfused with oxygenated aCSF at room temperature. As with plasticity experiments, the experimenter remained blind to subject strategy and turn direction. In each hemi-slice, up to two medium spiny neurons (MSNs) were patched: one dorsomedial and one dorsolateral. No more than two cells were obtained from the same animal in a given region. Cells were patched under visual guidance using IRDIC imaging (Zeiss Axioskop2 FS plus). Pipettes were fire-polished (Narishige MF-830) to a resistance of 4-7 MΩ, and filled with a potassium based internal solution (in mM: K-gluconate 132, KCl 10, NaCl 8, HEPES 10, Mg-ATP 3.56, Na-GTP 0.38, EGTA 0.1, Biocytin 0.77) of pH 7.3. Intracellular signals were collected in current clamp and filtered at 3 kHz using an EPC 10 amplifier and Patchmaster software (HEKA Electronik). Series resistance (6-15MΩ) was compensated 80%, but capacitance was not compensated. Cells were determined to be MSNs by their low resting membrane potential (near -80mV), rounded AHPs, and long latency to first action potential. Current-voltage (IV) and current-frequency (IF) curves were recorded from each cell using 400ms current injections. Because MSNs display strong inward rectification, their IV curves display distinct linear components at potentials negative and positive to rest. Therefore we analyzed two input resistance values for each cell by fitting a line to the IV curve at current injections of -500pA to -100pA (IRneg) and at 0pA to

+100pA (IRpos). More positive current injections were excluded from input resistance analysis to avoid contamination from action potential firing. Rheobase was the lowest current injection value eliciting an action potential, and latency was the time between onset of current injection and action potential peak at rheobase.

Morphology

MSNs were filled with biocytin through the patch pipette for 20 to 30 minutes during excitability measurements. Hemi-slices were then fixed in 4% paraformaldehyde overnight before removal to phosphate buffered saline (PBS). 350µm thick hemi-slices were stained using the biocytin staining protocol for thick slices (Marx et al., 2012). Briefly, after fixation and rinsing in PBS, slices were incubated in the Vectastain ABC kit (Vector Labs) overnight at 4°C. After further rinsing in PBS, slices were stained using the DAB kit (Vector labs) with the nickel addition. Slices were then rinsed in PBS and dried overnight in a humid chamber on gelatin coated slides. Finally, slices were slowly dehydrated in an ethanol series (25%, 35%, 45%, 55%, 65%, 75%, 85%, 95%, 100%) and cleared in Xylene. Eukitt mounting medium (Vector labs) was used for cover slipping.

Successfully stained neurons were reconstructed directly from the tissue. Neurons were fully reconstructed at 40x magnification without spines, and partially reconstructed (one branch) at 100x magnification to count spines. The branch selected for high magnification reconstruction was the primary dendritic branch with the most clearly identified spines. Reconstructions were done manually, i.e. a human reconstructor used a cursor to trace and mark visible structures on the monitor using the software Neurolucida (v7), while adjusting focus to move through the tissue in the z axis (depth).

Reconstructors were trained identically, and were blind to subjects' experimental condition.

Dendritic length, number of branch points, and spine density were each analyzed by path distance from the soma, as opposed to the more traditional Scholl (i.e. Euclidean) distance. Path distance measures distance from the soma when traveling along the dendrites. Within a bin of set *path* distance, the amount of dendritic length depends on the number of contributing dendrites, and thus depends on the number of branches and the length of branches present. Note that, unlike Scholl distance, the amount of dendritic length within a set path distance from the soma is unchanged by tortuosity.

Structure and spine density analysis were conducted in NeuroExplorer, and values were transferred to SAS for statistical analysis. Because of variability between reconstructors, randomly selected cells were reconstructed multiple times by different reconstructors; such repetition was distributed evenly among experimental conditions, and we included reconstructor as an independent factor in all analyses. In addition, care was taken so that all potential sub-groups (such as hemisphere relative to the learned turn) were represented within each training condition.

The untrained control group in the morphology section includes fully naïve rats which were never food restricted or regularly handled. As reported in results, naïve neuronal morphology measures are statistically indistinguishable from those of our habituated controls. Only habituated controls are used for all other sections of the study.

Analysis

Figures were made using IGOR (v6.1.2.1). Statistical analysis was carried out in SAS (v9.3, SAS Institute). The procedure GLM (general linear model) was used to carry out analysis of variance and repeated measures analysis of variance, and GLM contrast was used for post-hoc comparisons. The procedure FREQ was used to carry out chi square analyses. The procedure TEST was used to assess plasticity in habituated controls, and to compare plasticity across hemispheres. To compare plasticity across hemispheres, we calculated the difference between ipsilateral and contralateral plasticity (population spike amplitude relative to baseline averaged over the final 15 min after induction) both after 1^{st} and 3^{rd} induction period. This hemisphere comparison was applied only to animals for which both ipsilateral and contralateral data were collected using the same induction protocol and region. For plasticity graphs and statistical analysis, n is number of experiments, with not more than one experiment per slice, and not more than two identical treatments collected from the same animal. For excitability and morphology graphs and statistical analysis, n is number of cells, with not more than two cells (one medial and one lateral) per slice, and not more than two cells from the same region collected from the same animal. Tests where $P<0.05$ are reported as significant, and "trend" toward significance is mentioned for some P-values falling between 0.05 and 0.1. P-values for all described findings are given in the text or else in tables. Means are reported ± standard error of the mean (SEM), and in all graphs error bars illustrate SEM.

Results

T-maze strategy transition distinguishes early- and late-trained groups

To investigate the involvement of distinct striatal regions as learning progresses, we train rats in T-maze navigation (Fig 1A,B). Maze training transitions subjects through recognizable performance stages; in particular subjects demonstrate a *place* strategy during maze acquisition and a *response* strategy once maze navigation is an acquired skill (Tolman et al., 1946;Dunnett and Iversen, 1981).

To confirm the *place* to *response* transition, we examined strategy use during the final probe at both trained stages. There was a significant relationship between strategy at final probe and training stage ($X^2(1,N=52)=4.74$ $P=0.0295$), such that early-trained rats made greater use of a *place* strategy while late-trained rats predominantly demonstrated a *response* strategy (Fig 1B,C). Time to reach reward decreased markedly prior to the first probe (Fig 1D), suggesting rapid acquisition of the reward location. Frequency of rats' visual inspection of alternative choice arms prior to action selection, termed vicarious trial and error (VTE), was analyzed as this represents a behavioral correlate of *place* strategy use and attentive decision-making associated with dorsomedial striatal engagement (Schmidt et al., 2013). VTE was most frequent at the first probe, and declined across training and probes (Fig 1E). Both strategy during probe trial and frequency of VTE demonstrated strategy transition throughout the course of training.

Figure 1.

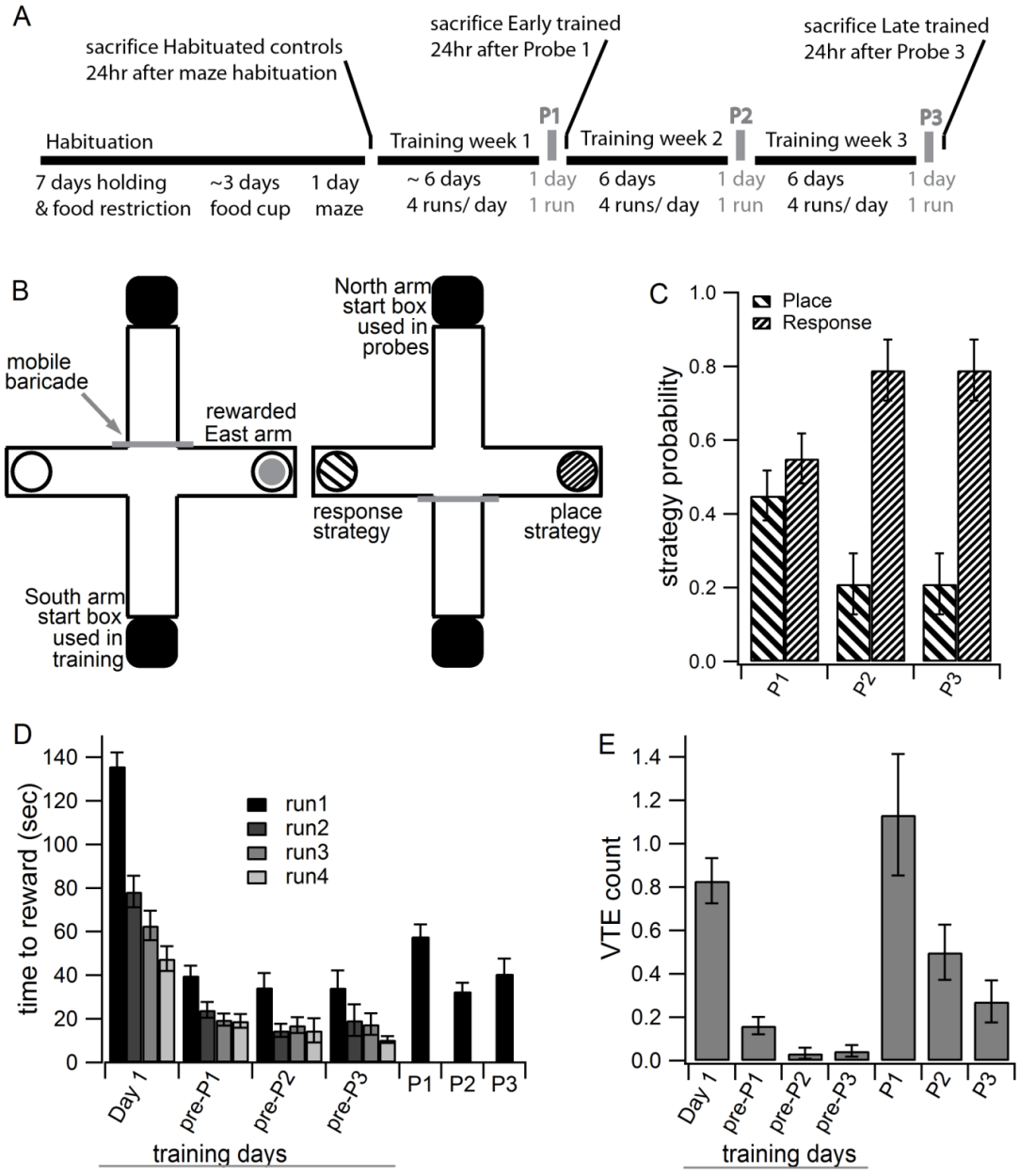

Figure 1. T-maze learning is accompanied by a strategy shift with skill acquisition. **A.** Schematic showing behavior timeline. **B.** T-maze diagram for training (left) and testing (right) when East arm is rewarded. Turning East is scored as *place* strategy, and turning West is scored as *response* strategy for animals rewarded during training for turning East. **C.** Early-trained animals are divided between *place* and *response* strategies; late-trained animals predominantly demonstrate the *response* strategy. **D-E.** Both time to reward (D)

and average VTE count (E) decrease with continued training, and both show elevation at probe trials relative to adjacent training days. For all panels, P1-P3 indicate probe1-probe3. Training days are indicated relative to probes such that "pre-P1" is the training day immediately preceding P1.

We assessed several alternative factors which could have influenced T-maze performance. We verified that experimental groups were not different preceding training, or at the time of first probe. There was a positive correlation between weight at sacrifice and both time to reach performance criteria and time to reach the reward on first and final probes (GLM, $F_{1,51}$=5.09 P=0.0284 days to criteria; $F_{1,51}$=4.88 P=0.0317 Probe 1 time to reward; $F_{1,51}$=4.46 P=0.0398 final Probe time to reward). This suggested that heavier rats were less food-motivated. However weight did not influence final probe strategy (GLM, $F_{1,51}$=2.55 P=0.1164). Habituated, early- and late-trained groups did not differ by weight at sacrifice (GLM, $F_{2,64}$=0.56 P=0.5731); early- and late-trained groups did not differ in days required to meet performance criteria (GLM, $F_{1,51}$=1.1 P=0.2997), or in strategy use at first probe (X^2(1,N=52)=0, P=1). Thus the only factor that predicted strategy at the time of sacrifice was training stage.

In summary our early- and late-trained groups differed significantly in navigation strategy. Early-trained rats employed a strategy associated with attentive performance and dorsomedial striatal engagement more frequently than late-trained animals, which more often employed a strategy linked to skilled performance and dorsolateral striatal engagement. We proceeded to examine diverse neuronal measurements across sub-regions and training-stages to test for physiological differences corresponding to these behaviors.

Striatal changes with learning

A recent study showed motor skill learning alters striatal plasticity (Yin et al., 2009), and here we build on this work by evaluating subregion specific changes in plasticity relative to the learned turn, by examining LTP alongside LTD, and by examining morphology as well as excitability in neurons after training. Because the T-maze training is a lateralized task (each rat learns to seek food on only one side of the maze), we assessed changes in striatal synaptic plasticity not only in dorsomedial and dorsolateral subregions during early versus late stages of learning, but also in hemispheres both ipsilateral and contralateral to the rewarded turn (Fig 2A). We assessed striatal plasticity and MSN excitability through extracellular and whole cell recordings, respectively, and examined morphology from reconstructions of those MSNs patched for excitability measurement.

Synaptic Plasticity

We measured change in population spike amplitude to assess corticostriatal synaptic plasticity in *ex vivo* brain slice, similar to others (Yin et al., 2009;Akopian et al., 2000;Adermark et al., 2011). Plasticity was measured in response to a series of inductions repeated at 30 minute intervals. In order to identify plasticity modulation with maze learning, we compared evoked plasticity among habituated and trained groups.

Dorsomedial LTP magnitude was reduced in early-trained rats in the contralateral hemisphere. Habituated controls exhibited robust dorsomedial LTP in response to theta-burst stimulation (138±8% 85-90min post-induction; T_9=4.4 P=0.0012). Statistical analysis demonstrated that training stage modified LTP exclusively within the hemisphere

contralateral to the learned turn (GLM repeated $F_{2,31}$=4.55 P=0.0185 contralateral, $F_{2,33}$=1.04 P=0.3617 ipsilateral). Post hoc time-matched comparison to habituated controls showed contralateral LTP magnitude was significantly reduced only for early-trained (P=0.0055) and not late-trained (P=0.2215) groups. As illustrated in Fig 2B, the same theta-burst stimulation which produced pronounced potentiation in habituated controls instead evoked transient depression in the contralateral hemisphere of early-trained rats. Note that in the ipsilateral hemisphere the early-trained group exhibited a transient depression in population spike amplitude immediately following induction, indicated by a within-subjects time x stage interaction (GLM repeated $F_{34,561}$=1.56 P=0.0246 stage x time; post hoc vs. habituated: P=0.0373 early, P=0.9748 late). However, in the ipsilateral hemisphere this transient depression was only evident after the 2nd and 3rd inductions, and the final magnitude of LTP was not significantly altered (Fig2 B). Habituated and late-trained groups did not differ in evoked LTP in either hemisphere. Comparing plasticity across hemispheres within each rat permits each animal to serve as its own control, though the sample size is lower because we were not able to collect contralateral and ipsilateral recordings for each subject. Nonetheless, comparison across hemispheres (Table 1) generally agrees with the repeated measures analysis. The hemispheric difference in dorsomedial LTP for early trained subjects is consistent for both induction periods, though not reaching significance due to reduced n. The hemispheric difference in dorsomedial LTP for late trained subjects has too few animals to say anything meaningful.

Figure 2

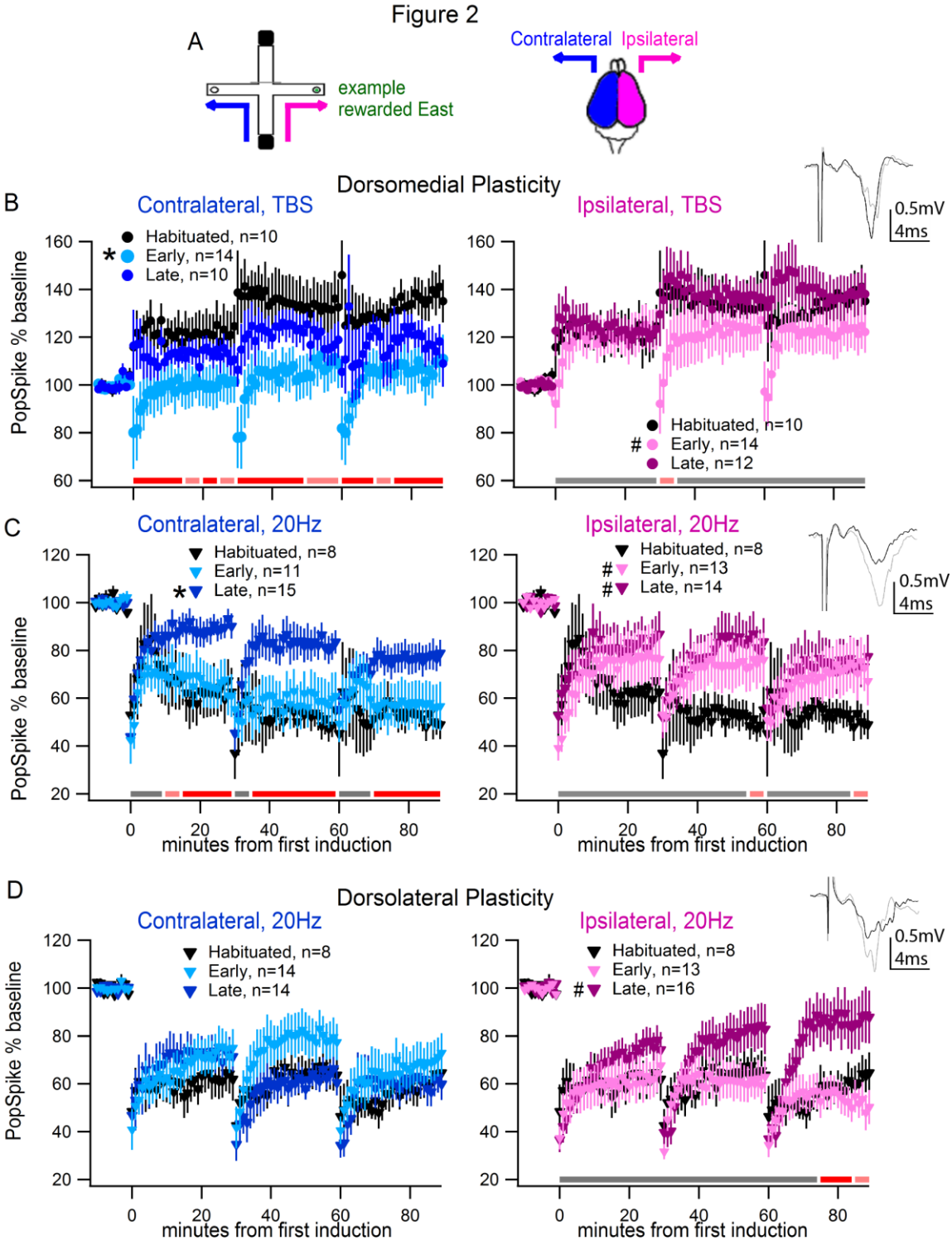
A
example
rewarded East
Contralateral
Ipsilateral
Dorsomedial Plasticity
B
Contralateral, TBS
Habituated, n=10
Early, n=14
Late, n=10
Ipsilateral, TBS
Habituated, n=10
Early, n=14
Late, n=12
0.5mV
4ms
PopSpike % baseline
C
Contralateral, 20Hz
Habituated, n=8
Early, n=11
Late, n=15
Ipsilateral, 20Hz
Habituated, n=8
Early, n=13
Late, n=14
minutes from first induction
D
Dorsolateral Plasticity
Contralateral, 20Hz
Habituated, n=8
Early, n=14
Late, n=14
Ipsilateral, 20Hz
Habituated, n=8
Early, n=13
Late, n=16

Figure 2. Corticostriatal plasticity changes with T-maze learning. **A.** Diagram color-coding brain hemispheres relative to the trained turn (blue contralateral, magenta ipsilateral; this hemisphere color-coding is used for panels B-D and Fig 3-6. In addition, light colors indicate early training, dark colors report late training in B-D and Fig 3-6). **B.** Dorsomedial LTP: Contralateral change appears early but not late in training. Ipsilateral LTP is unchanged. **C.** Dorsomedial LTD: Contralateral change appears late but not early in training. Ipsilateral change appears both early and late in training. **D.** Dorsolateral LTD: Contralateral LTD is unchanged. Ipsilateral change appears late but not early in training. For all panels, asterisks indicate the group(s) showing plasticity modulated by training-stage alone ($P<0.05$), and # indicates groups showing modulation by stage*time ($P<0.05$). Colored bars illustrate post-hoc comparison (GLM contrast) to habituated controls within time-matched five minute windows ($P<0.05$ red, $0.1\geq P\geq 0.05$ pink, $P>0.1$ gray). Example traces representing each subregional plasticity protocol in habituated controls appear at right (gray baseline, black post-induction).

Table 1. Inter-hemisphere plasticity difference. Effect size is ipsilateral – contralateral of %increase in population spike over baseline for animals which had both ipsilateral and contralateral measurements in same region and using same induction protocol. *indicates $P < 0.05$ for plasticity difference.

Table 1

	Early trained		Late trained	
	1st induction	3rd induction	1st induction	3rd induction
DM LTP	df=9 P=0.1408 effect size=23	df=9 P=0.3177 effect size=18	df=4 P=0.353 effect size=7	df=4 P=0.118 effect size=21
DM LTD	df=9 P=0.8905 effect size=-2	df=9 P=0.5492 effect size=5	df=8 P=0.4368 effect size=-0.11	df=8 P=0.9501 effect size=0.01
DL LTD	df=8 P=0.2023 effect size=-0.17	df=8 P=0.1115 effect size=-0.24	df=9 P=0.2234 effect size=0.14	df=9 *P=0.044 effect size=0.40

Dorsomedial LTD magnitude was reduced in both hemispheres by late training. Habituated controls exhibited robust dorsomedial LTD in response to 20Hz stimulation (50±5% 85-90min post-induction; $T_7=9.3$ $P<0.0001$). Statistical analysis confirmed a significant main effect of training stage within the hemisphere contralateral to the learned

turn (Fig 2C; GLM repeated $F_{2,31}$=3.78 P=0.0339 contralateral, $F_{2,32}$=0.65 P=0.5268 ipsilateral). Post hoc time-matched comparison to habituated controls showed that contralateral LTD was unchanged for early-trained rats (P=0.5079), and was reduced for late-trained rats (P=0.0173). In addition, within the ipsilateral hemisphere, we found a significant within-subjects time x stage interaction indicating reduced LTD compared to habituated controls for both training stages (GLM repeated $F_{34,527}$=1.51 P=0.0338 stage x time; post hoc vs. habituated: P=0.0034 early, P=0.0031 late, Fig 2C). Despite the reduced LTD ipsilaterally at some time points for early trained animals, the comparison across hemispheres does not support lateralization in dorsomedial LTD for early trained animals (Table 1). On the other hand, the comparison across hemispheres (Table 1) confirms no difference in dorsomedial LTD for late-trained animals.

Dorsolateral LTD magnitude was reduced by late training exclusively in the hemisphere ipsilateral to the learned turn. Habituated controls exhibited robust dorsolateral LTD in response to 20Hz stimulation (62.2±7% 85-90min post-induction; T_9=5.3 P=0.0005). Here training stage did not produce altered LTD immediately following induction. Instead, a significant time x stage effect exclusively within the ipsilateral hemisphere (Fig 2D; GLM repeated $F_{34,595}$=3.0 P<0.0001 time x stage) demonstrated a marked reduction in the persistence of LTD. Post hoc analysis indicated difference from controls is restricted to the late-trained group ($F_{17,595}$=0.42 P=0.9810 early, $F_{17,595}$=3.84 P<0.0001 late). Significant inter-hemispheric difference in late-trained dorsolateral LTD is supported by the within subjects comparison (Table 1).

Because the early-trained rats were evenly split between place and response strategy, we further analyzed whether response strategy was a better predictor of plasticity than training stage. First, we performed the repeated measures analysis using strategy instead of stage (with strategy = NA for habituated controls). Neither strategy nor the strategy by time interaction term was significant for any brain region or induction protocol. Then, we compared evoked plasticity (change from baseline averaged over the final 15 minutes) among three groups: early-trained rats using place strategy, early-trained rats using response strategy, and late-trained rats using response strategy (Fig 3). Late-trained rats using a place strategy were excluded because of insufficient numbers. Again, strategy was not a significant predictor of plasticity, though for dorsolateral LTD within the contralateral hemisphere a trend toward significance arises from difference between early-trained animals using place versus response strategies (Fig 3C; GLM, $F_{2,22}$=2.88 P=0.0773). In summary, this analysis suggests that training stage is a better predictor of the change in plasticity than is response strategy.

Figure 3.

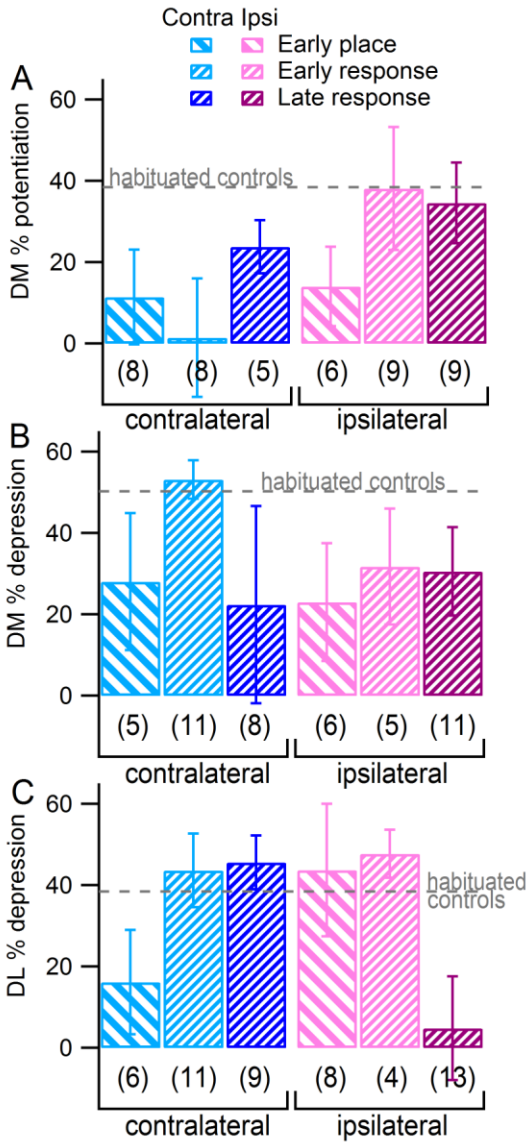

Figure 3. Strategy does not predict plasticity outcome. Percent potentiation or depression in the final 15 minutes of plasticity experiments is graphed by final probe strategy for trained animals, with n indicated below each group. For comparison, a dashed line illustrates mean percent plasticity in habituated controls. **A.** Dorsomedial LTP. **B.** Dorsomedial LTD. **C.** Dorsolateral LTD.

The previous analyses grouped together right-turning and left-turning rats. To verify that the changes in plasticity with training appeared for both turn directions, we repeated the GLM repeated measures analysis by training stage both for rats rewarded for turning East and for rats rewarded for turning West. Table 2 shows that the effect of training stage on both LTP and LTD in the contralateral hemisphere of dorsomedial striatum was observed for both turn directions. For LTP, training stage significantly influences plasticity contralaterally but not ipsilaterally; both for rats rewarded for turning East and (at trend level) for those rewarded for turning West. For dorsomedial LTD, a contralateral stage effect is apparent in rats rewarded for turning East (at trend level) and also in those rewarded for turning West. In contrast to this consistency for contralateral measurements, ipsilateral LTD in both dorsomedial and dorsolateral striatum shows a significant training stage effect exclusively within rats trained to turn West, which is absent in East-rewarded rats. Should this finding be replicated, it would suggest some degree of lateralization of the task within the striatum. Other than these two groups, plasticity findings derived independently within East- or West-rewarded groups show good correspondence to results derived from all subjects.

Table 2. Plasticity changes are similar in both East and West rewarded rats. Trend level significance ($0.05 < P < 0.1$) in training stage is indicated by ~, $P < 0.05$ indicated by *.

Table 2

	Included subjects	Contralateral	Ipsilateral
DM LTP	All rats	$F_{2,31}$=4.55 *P=0.0185	$F_{2,33}$=1.04 P=0.3617
	rewarded East	$F_{2,19}$=3.92 *P=0.0377	$F_{2,18}$=0.27 P=0.7646
	rewarded West	$F_{2,19}$=2.75 ˜P=0.0896	$F_{2,22}$=1.11 P=0.3465
DM LTD	All rats	$F_{2,31}$=3.78 *P=0.0339	$F_{2,32}$=0.65 P=0.5268
	rewarded East	$F_{2,21}$=3.26 ˜P=0.0586	$F_{2,20}$=1.98 P=0.1639
	rewarded West	$F_{2,15}$=4.77 *P=0.0250	$F_{2,17}$=4.2 *P=0.0330
DL LTD	All rats	$F_{2,35}$=0.6 P=0.5564	$F_{2,36}$=2.87 ˜P=0.0696
	rewarded East	$F_{2,20}$=0.7 P=0.5065	$F_{2,18}$=0.04 P=0.9628
	rewarded West	$F_{2,22}$=0.36 P=0.7021	$F_{2,25}$=7.91 *P=0.0022

Together our findings reveal novel patterns coupling learning stages with altered plasticity relative to the learned turn. Hemisphere-specific changes in dorsomedial plasticity align with early-training, at which point reduced (eliminated) LTP is observed contralaterally, without a change in LTD, suggesting that LTP and LTD are modified independently. Hemisphere-specific change in dorsolateral plasticity aligns with late-training, at which point reduced LTD is observed in the ipsilateral striatum. Thus hemisphere specific (i.e. turn-relative) plasticity differences are present dorsomedially early in training and dorsolaterally late in training.

Excitability

In addition to synaptic change, plasticity in intrinsic excitability may be integral to learning. Altered neuronal excitability can directly facilitate transmission of signals in support of learned behavior, and may provide a metaplastic backdrop modulating synaptic plasticity's direction or impact (Zhang and Linden, 2003;Abraham,

2008;Rogerson et al., 2014). Recognizing that intrinsic plasticity changes in striatal MSNs during T-maze learning could be important for learning, we assess both population and single cell excitability measures.

Extracellular input output (IO) curves related strength of afferent depolarization to striatal population spike amplitude and were collected preceding induction for plasticity experiments. Statistical analysis of extracellular IO curves within habituated control rats revealed no difference between striatal regions (GLM $F_{1,50}$=0.24,P=0.62). Comparing training stages, we found a significant training effect in the IO curve shape ($F_{2,271}$=5.67,P=0.0039). Specifically, peak output was smaller in dorsomedial striatum in late-trained rats, in the hemisphere contralateral to the learned turn (Fig 4A; post hoc comparison to habituated control, P=0.0009). No difference from controls was detected in early-trained rats, in late-trained rats ipsilateral to the learned turn, nor in any dorsolateral group. Importantly, plasticity results were not due to difference in extracellular responsiveness as half-maximal current from IO curves was used for all plasticity experiments, and did not differ among groups.

Recording from MSNs in whole-cell mode, we examined intrinsic electrophysiological properties in single neurons across training groups and striatal regions. Specifically, we measured resting membrane potential (RMP), rheobase, input resistance both positive (IRpos) and negative (IRneg) to RMP, evoked spiking, and spike latency during somatic current injection. We examined habituated controls for inter-regional differences prior to learning the T-maze, and found a small but significant difference in RMP (Fig 4B, GLM $F_{1,22}$=5.04 P=0.0356; DM -81.18±0.6 mV, DL -

79.89±0.4 mV) which disappeared with training. No other whole cell measure differed between regions for habituated controls. Analysis by region and across training stages showed significant changes in RMP with training for dorsomedial striatum, such that MSNs from early-trained animals are more depolarized at rest, and return to control-matched RMP by late training (Fig 4B; GLM $F_{2,53}$=4.09 P=0.0226; RMP(mV): -81.18±0.6 habituated, -78.99±0.6 early, -81.67±1.1 late). RMP did not change for dorsolateral cells.

Several complimentary, inter-regional differences in excitability measures indicate dorsomedial excitability was increased relative to dorsolateral in early-trained animals; each of these differences was restricted to the contralateral hemisphere. When contralateral and ipsilateral hemispheres were considered together, we found that inter-regional (DM-DL) difference – which is absent in untrained animals - appears in rheobase (Fig 4C) and in IRpos (Fig 4D) and is significantly modulated with training stage ($F_{2,40}$=3.37 P=0.0448 rheobase; $F_{2,40}$=3.28 P=0.0487 IRpos). Reduced rheobase and increased input resistance dorsomedially contributed to a trending left-shift in the current-frequency (IF) curve for the dorsomedial relative to dorsolateral striatum in early training (Fig 4E; GLM $F_{2,40}$= 3.23 P=0.0506 IF half max). Analysis by hemisphere relative to the learned turn revealed that each of these inter-regional differences was highly significant for the contralateral hemisphere (Fig 4C-E; GLM $F_{1,21}$= 8.5 P=0.0086 rheobase; $F_{1,21}$=8.36 P=0.009 IRpos; $F_{1,21}$=9.95 P=0.005 IF half max) but not for the ipsilateral hemisphere (GLM $F_{1,22}$= 3.06 P=0.0949 rheobase; $F_{1,22}$=3.58 P=0.0725 IRpos; $F_{1,22}$=3.88 P=0.0621 IF half max). By late training, inter-regional differences were absent within and

across hemispheres. No regional or training-related change was detected for spike latency. Excitability measures are summarized in Table 3.

Figure 4.

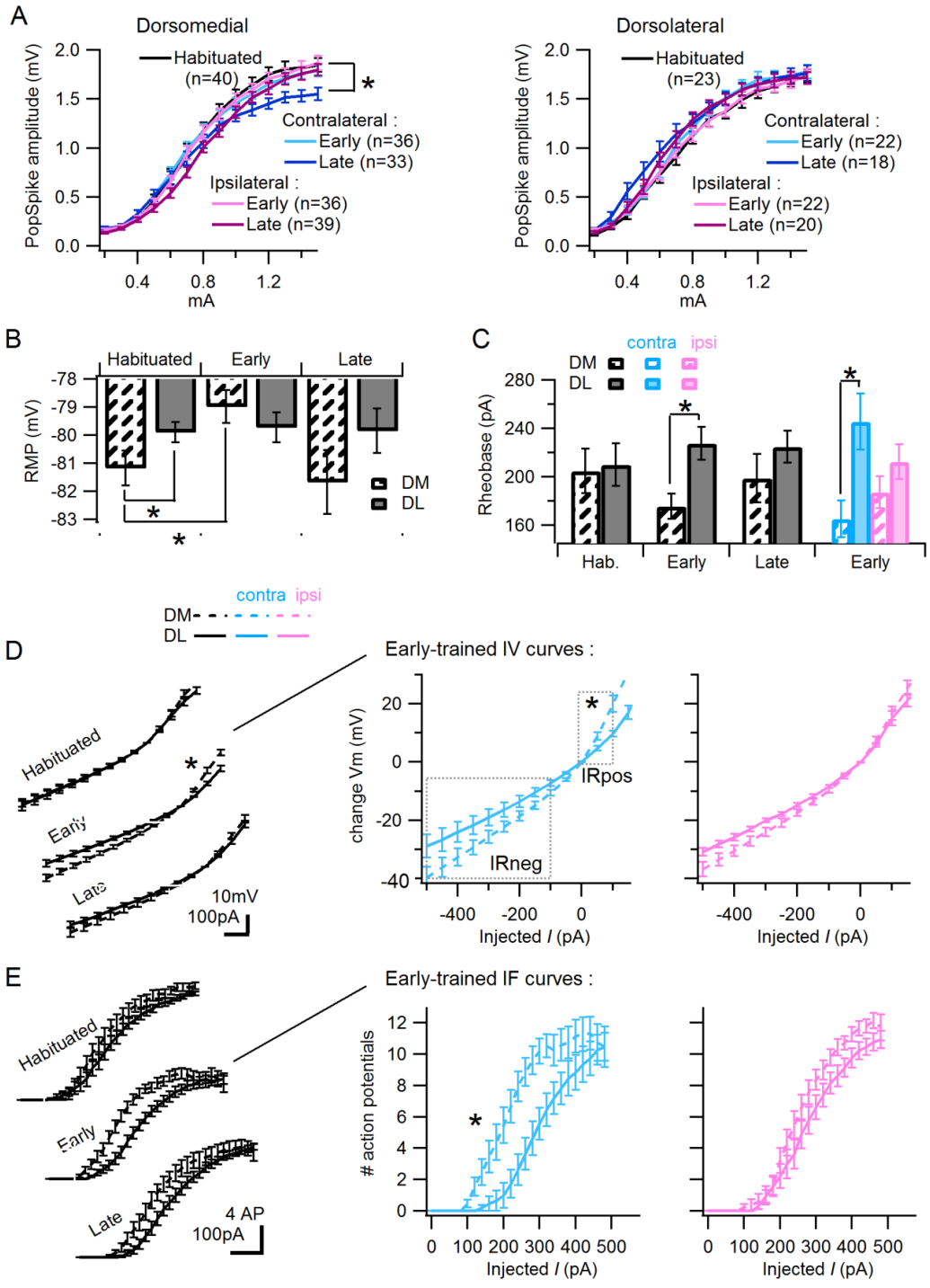

Figure 4. Intrinsic excitability is regionally modulated with training stage. **A.** Extracellular IO curves are identical for all groups in DM striatum except the contralateral, late-trained group, in which the peak is reduced. No change in IO curves was detected in DL striatum. **B.** RMP differs regionally in MSNs from habituated control rats. A significant increase (less hyperpolarization) of dorsomedial RMP is seen with early-training. **C.** Rheobase is reduced in the dorsomedial relative to dorsolateral region during early-training. **D.** The slope of IV curves is used to measure input resistance, both from -500 to -100pA (IRneg) and 0 to +100pA (IRpos). IRpos is significantly increased in the dorsomedial relative to dorsolateral region during early-training. **E.** Dorsomedial IF curve is left-shifted relative to dorsolateral during early-training. Significant differences in C-E are due to change in the contralateral hemisphere, as illustrated at right in color. Parallel analysis of late-trained groups by hemisphere shows no difference. For all panels, asterisks correspond to $P<0.05$, detailed in results. For whole-cell measures, DM n = 12 habituated, 29 early, 16 late; DL n = 12 habituated, 24 early, 15 late.

Table 3. Excitability differs regionally with early-training. Means and SEM (corresponding to Figure 4 D-E) are given for rheobase, input resistance positive (IRpos) and negative (IRneg) to rest, and for IF curve current injection eliciting half maximal firing.

Table 3

	Overall		Contralateral		Ipsilateral	
	DM (26)	DL (20)	DM (13)	DL (9)	DM (13)	DL (11)
Rheobase (pA)	172.8±11	233±15	164.62±17	181.67±13	188.57±24	216.36±15
IRneg (MΩ)	69.73±4	55.84±4	71.38±6	59.14±7	67.94±5	53.14±4
IRpos (MΩ)	193.9±16	121.06±12	211.15±25	111.24±21	175.22±19	129.09±15
IF ½max (pA)	208.07±11	278.13±16	200.47±17	301.02±29	216.31±13	259.41±17

In summary, changes in intrinsic excitability measures combine to show transient enhancement in excitability for dorsomedial relative to dorsolateral striatum. This enhancement emerges during early learning and dissipates with prolonged training. Importantly, inter-regional excitability differences emerge in a turn-relative pattern

(exclusive to the hemisphere contralateral to the learned turn), connecting intrinsic excitability modulation to behavioral modification with learning.

Morphology

Morphological changes such as new spine growth are reported with learning (Knott and Holtmaat, 2008). We therefore reconstructed the same MSNs from which whole-cell excitability measures were collected in order to investigate potential morphological covariance with learning. For each reconstructed neuron, morphological measurement included spine density, number of primary dendrites, total dendritic length, and the number of dendritic branch points as a function of path distance (as opposed to Scholl distance) from the soma. Spine density (counted from images at 100x magnification; Fig 5B), number of branch points, and dendritic length were analyzed in 20µm bins out to 120µm from the soma; beyond this distance the number of usable samples falls off.

Data on spine density shows that, for all conditions, spine density is low near the soma and rises to peak around 60µm as has been reported for MSNs (Berlanga et al., 2011). The dependence of spine density on distance from the soma is statistically significant and spine density also varies by reconstructor (GLM repeated, $F_{5,255}$=97.07 P<0.0001 distance; $F_{10,225}$=7.63 P<0.0001 reconstructor*distance), but spine density does not differ by training stage ($F_{10,225}$=0.46 P=0.8466 stage*distance). The interaction term reconstructor by stage is not significant, suggesting that difference in reconstructor style does not obscure a difference due to training. Spine density also does not differ by hemisphere (GLM repeated, $F_{10,255}$=0.9727 P=0.9266 hemisphere* distance) or by striatal

region (GLM repeated, $F_{5,260}$=0.98 P=0.4072 region* distance). Figure 5 shows spine density by training stage, distance, and either hemisphere (Fig 5D,E, collapsed across region) or region (Fig 5F,G, collapsed across hemisphere). Our results suggest T-maze learning occurs without persistent alteration in striatal spine density.

A remarkable change in dendritic arbor complexity with training is evident through analysis of 40x reconstructions (Fig 5C). Changes with training are illustrated in figure 5A by representative dendrograms and reconstructions from an untrained and from a trained animal (example cells are habituated and late-trained, respectively), which shows a reduced number of dendrites for the trained animal. Cumulative dendritic length varies by training stage (GLM, $F_{2,54}$=14.21 P<.0001), and neither reconstructor nor the interaction term training stage*reconstructor are significant (Type III SS, $F_{4,54}$=1.85 P=0.1378 reconstructor; $F_{8,54}$=1.11 P=0.379 reconstructor*stage), indicating that reconstructor difference does not produce the training stage effect. Relative to controls, cumulative dendritic length is reduced in trained animals, but shows no difference between early- and late-trained groups (GLM contrast, P=0.0002 early vs. untrained, P=0.0047 late vs. untrained, P=0.329 early vs. late trained). Table 4 summarizes cumulative dendritic length (which encompasses the influence of reduced branches) by region, stage, and hemisphere. For each of the four groups defined by region and hemisphere, separate GLM of cumulative dendritic length by training stage were performed. All but the dorsolateral contralateral region showed a significant training effect (P<0.0403), and post hoc contrast indicates difference from untrained, but not between early- and late-trained except in the dorsomedial ipsilateral region (Table 4).

Figure 5.

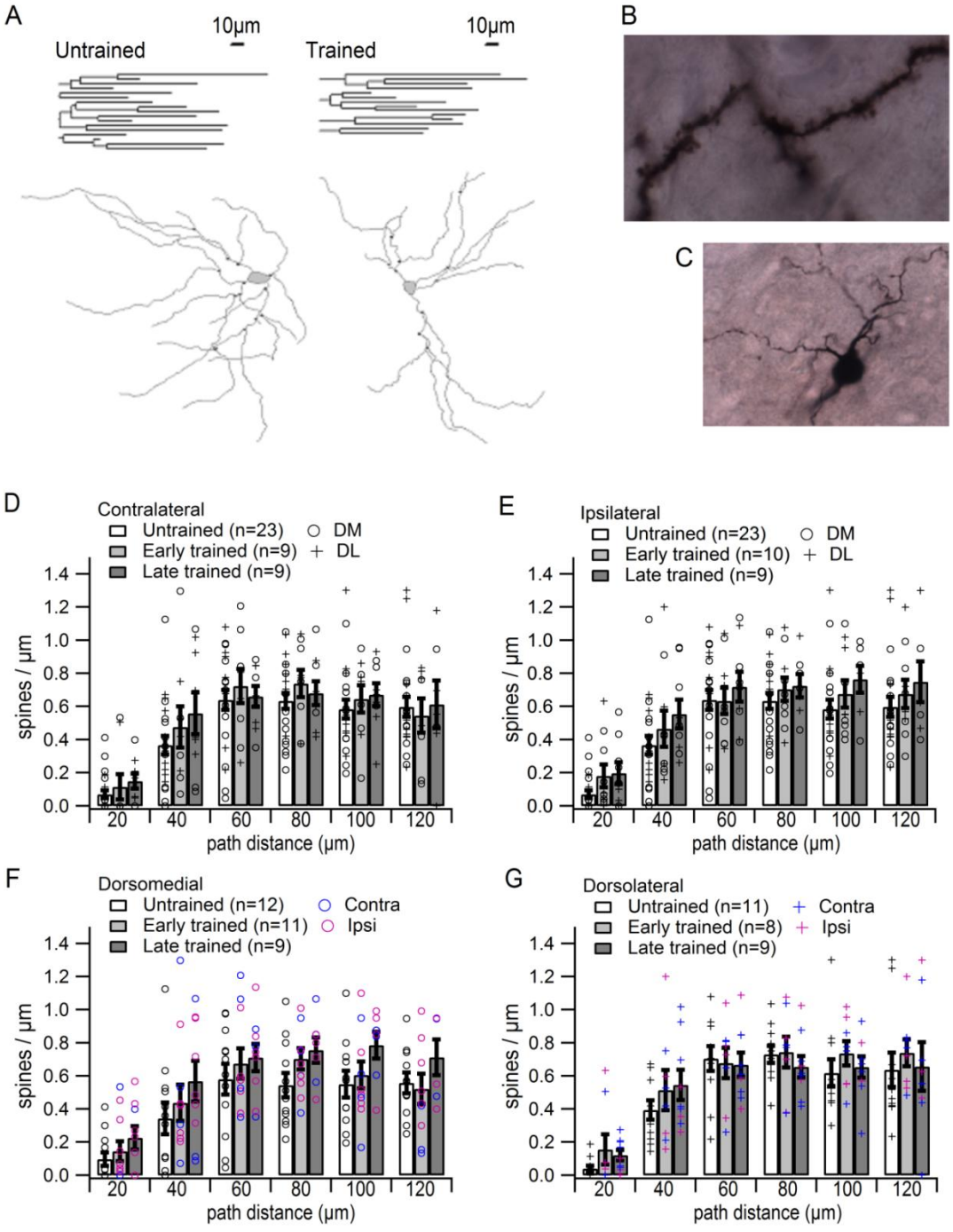

Figure 5. Medium spiny neuron dendrites, but not spines, are changed with training. **A.** Representative dendrograms and reconstructions with marked branch points from untrained and trained animals. Scale bars relate to both dendrograms and to

reconstructions. **B.** A representative MSN branch reconstructed at 100x. **C.** Several branches reconstructed at 40x. **D-G.** Spine density is unchanged with training (D-E) in both hemispheres, and (F-G) in both subregions.

Table 4. Cumulative Dendritic Length. Means and SEM (μm) are given, and associated P-values indicate difference from untrained controls. *indicates statistical significance of $P < 0.05$ in post hoc contrast to untrained controls. # indicates a significance difference (P=0.0107) between MSNs from early and late trained rats in the ipsilateral hemisphere of dorsomedial striatum. n is number of reconstructed neurons.

Table 4

	Untrained	Trained	
	NA	Contralateral	Ipsilateral
DM	1311.5± 122 (n=11)	850.3±97 early (n=5); *P=0.024 932.6±56 late (n=3); *P=0.0261	672.7±65 # early (n=5); *P=0.0001 962.2±55 # late (n=5); *P=0.014
DL	1165.7± 118 (n=12)	1050.7±30 early (n=4); P=0.37 824.1±124 late (n=5); P=0.1265	750.2±147 early (n=4); *P=0.0201 755.8±101 late (n=5); *P=0.0138

More detailed analysis of dendritic arbors by 20μm distance bins from the soma confirms that training stage influences dendritic length (GLM repeated, $F_{5,260}=42.88$ P<.0001) and also number of branch points ($F_{5,260}=53.93$ P<.0001). Post hoc comparison to controls at various path distances shows fewer branch points in trained animals between 21 and 120 μm from the soma (Fig 6A-D; $P < 0.05$ at each distance), with no difference between early- and late-trained animals (P>0.1516). Similarly, dendritic length is reduced in trained animals between 21 and 120 μm from the soma (Fig 6E-H) with no difference between early- and late-trained animals (P>0.05). Due to limited sample size, we analyzed effect of region or hemisphere separately while collapsing across the other factor. Fig 6 shows that, when collapsing across hemisphere, there is no difference

between DM and DL for number of branch points (GLM repeated, $F_{1,33}$=0.11 P=0.7469) or dendritic length (GLM repeated, $F_{1,33}$=0.06 P=0.8066). When collapsing across region, hemispheric difference in number of branch points is not significant (GLM repeated, $F_{1,33}$=3.84 P=0.0584), but the hemispheric difference in dendritic length is significant (GLM repeated, $F_{1,33}$=4.92 P=0.0336) such that training related reductions in these measures are more pronounced ipsilaterally (Fig 6E,F). The number of primary dendrites is unchanged across groups, as indicated by no difference in either dendritic length or branch point number 0-20µm from the soma ($F_{2,54}$=1.83 P=0.1706, $F_{2,54}$=0.76, P=0.4729, respectively).

Figure 6.

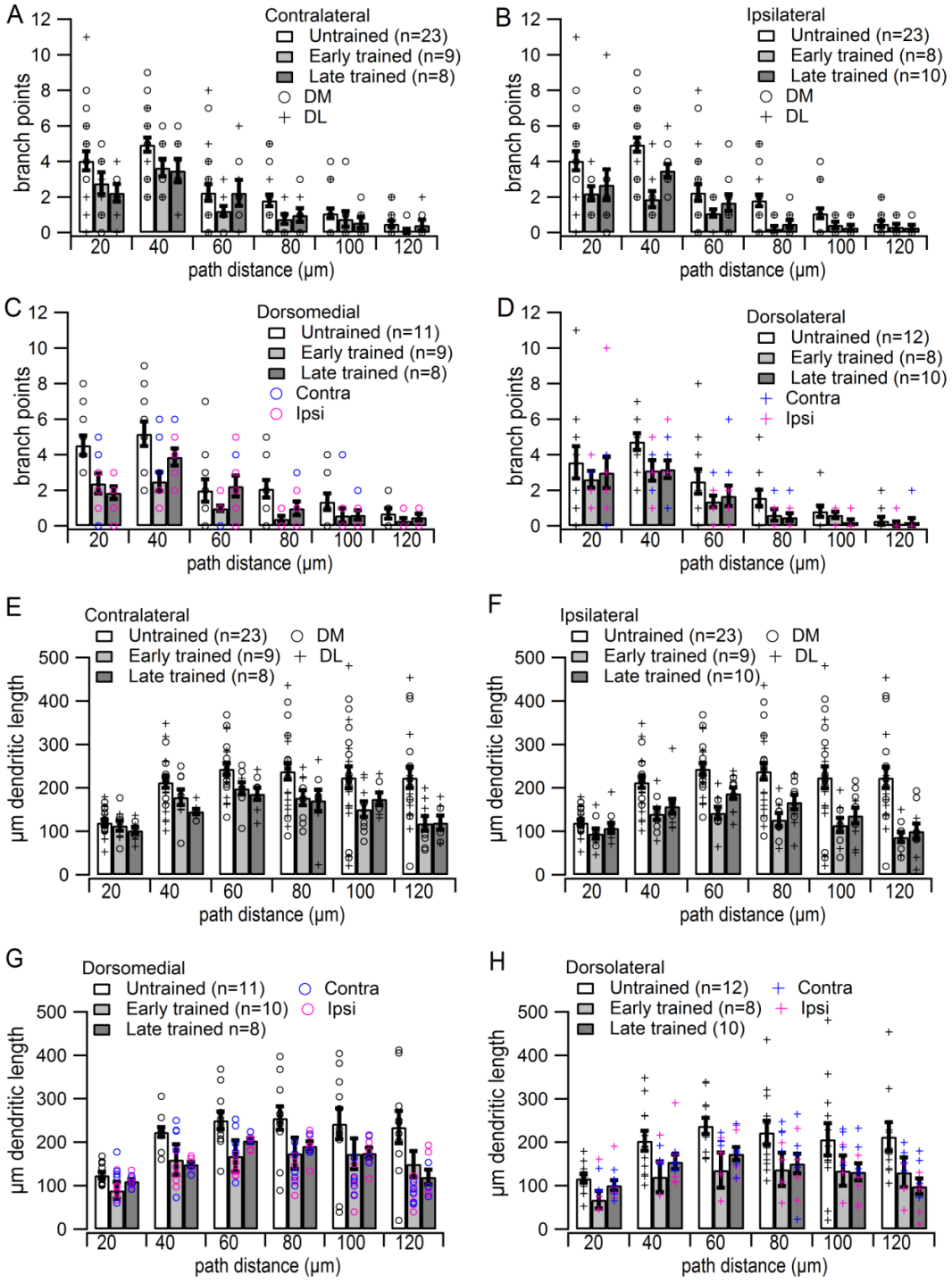
A
Contralateral
Untrained (n=23)
Early trained (n=9)
Late trained (n=8)
DM
DL
branch points
path distance (µm)
B
Ipsilateral
Untrained (n=23)
Early trained (n=8)
Late trained (n=10)
DM
DL
branch points
path distance (µm)
C
Dorsomedial
Untrained (n=11)
Early trained (n=9)
Late trained (n=8)
Contra
Ipsi
branch points
path distance (µm)
D
Dorsolateral
Untrained (n=12)
Early trained (n=8)
Late trained (n=10)
Contra
Ipsi
branch points
path distance (µm)
E
Contralateral
Untrained (n=23)
Early trained (n=9)
Late trained (n=8)
DM
DL
µm dendritic length
path distance (µm)
F
Ipsilateral
Untrained (n=23)
Early trained (n=9)
Late trained (n=10)
DM
DL
µm dendritic length
path distance (µm)
G
Dorsomedial
Untrained (n=11)
Early trained (n=10)
Late trained n=8)
Contra
Ipsi
µm dendritic length
path distance (µm)
H
Dorsolateral
Untrained (n=12)
Early trained (n=8)
Late trained (10)
Contra
Ipsi
µm dendritic length
path distance (µm)

Figure 6. Dendritic complexity is reduced in trained animals. **A-D.** Branch points are reduced in trained animals (A-B) in both hemispheres, and (C-D) in both subregions. **E-H.** Dendritic length is reduced in trained animals (E-F) in both hemispheres, and (G-H) in both subregions.

The lack of difference between early- and late-trained rats is in marked contrast with the electrophysiology data. To ensure that the trained effect in morphology was not due to a difference in handling between trained and untrained animals, the morphological analysis presented above includes an additional control group: neurons from naïve rats that were never food restricted or regularly handled. Analysis shows that morphology is statistically indistinguishable when comparing habituated controls and naïve rats (GLM repeated: spine density $F_{5,100}$=0.25 P=0.9385 stage*distance; branch count $F_{5,90}$=1.28 P=0.28 stage*distance; dendritic length $F_{5,90}$=1.32 P=0.26 stage*distance). In terms of handling and length of time in food restriction, the habituated and naïve controls are quite different (0 days for naïve controls vs. 11±1.1 days for habituated controls), while habituated controls and the early-trained animals are quite similar (differing by roughly 6 days). Early- and late-trained rats differ considerably more in time spent experiencing handling and food restriction (roughly 13 days). This strongly suggests that difference in experience outside of maze training cannot explain the morphological changes we find in trained rats. In summary, the morphology results reveal a change in the dendritic arbors of adult MSNs, which is specific to training, but which does not distinguish early- and late-trained groups.

Discussion

We analyzed bidirectional synaptic plasticity, population and single-cell excitability, and morphology from medium spiny neurons to investigate the contributions of anatomical and task-defined dorsal striatal regions to maze learning. Our data reveal independently altered LTP and LTD, as well as changes in MSN excitability and dendritic remodeling not previously reported with learning. Importantly, this is the first study describing dynamic lateralization in striatal plasticity relative to the direction an animal is trained to turn.

Consistent with previous reports, our early-trained group shows variability in T-maze strategy selection, while a response strategy predominates in more extensively trained rats (Packard, 1999;Yin and Knowlton, 2004;Lex et al., 2011). Fittingly, VTEs which suggest heightened spatial awareness and deliberative decision-making (Papale et al., 2012;Schmidt et al., 2013) are most frequent early in training. Reduced VTE, swift maze completion, and a predominant *response* strategy indicate progress toward habitual performance with late-training. Recordings *in vivo* show that modulation in MSN activity that corresponds to learning success emerges dorsomedially first, and develops dorsolaterally later in training (Yin et al., 2009;Thorn et al., 2010). Furthermore, lesion studies reveal that dorsomedial striatum (working with the hippocampus) is required for goal directed behavior and spatially attentive learning (Moussa et al., 2011;Lee et al., 2014), whereas dorsolateral striatum is required for automatic responses to stimuli and habit development with over-training (Yin and Knowlton, 2004). Therefore our subjects' shift from spatially attentive toward automatic or habitual performance suggests that a

shift from dorsomedial to dorsolateral engagement distinguishes early- and late-trained groups.

Hemisphere specific findings are consistent with prior studies demonstrating striatal engagement and plasticity during lateralized behavior. Unilateral striatal lesions generate turning toward the lesioned hemisphere (Ungerstedt et al., 1969), and MSN firing is negatively correlated with ipsilateral (Bryden et al., 2012) and positively correlated with contralateral turning behavior (Cui et al., 2013). NMDA subunit composition is modified in opposite directions within striatal hemispheres relative to the reaching limb (Kent et al., 2013). These studies confirm the importance of lateralization in our results.

Our results show agreement with Yin et al., 2009, the only other study to illustrate change in evoked dorsal striatal plasticity with skill learning. Their study found a depressed AMPA:NMDA ratio dorsolaterally with over-training, which complements our finding reduced LTD (ipsilateral) in this subregion with late-training. Contralaterally, we see the same late-trained flattening of dorsomedial IO curves and the same dorsomedial reduction in evoked LTD without dorsolateral reduction. Ipsilaterally we find the same dorsomedial LTD reduction but without the flattened IO curves. In contrast to Yin et al., 2009, dorsolateral LTD is reduced rather than enhanced relative to controls. This divergence in results may be attributed to our use of habituated controls while those in Yin et al., 2009 were naïve, representing subtly different time points on the spectrum from task-naïve to over-trained. In addition, locomotor and cognitive demands distinguish T-maze learning.

Our results do not distinguish between direct pathway and indirect pathway MSNs, although it is recognized that these neurons utilize different plasticity mechanisms. For example, the intracellular cascades and neuromodulation critical to bidirectional plasticity differ between direct and indirect pathway MSNs (Ding et al., 2010;Shen et al., 2008;Lerner and Kreitzer, 2012). Though molecular mechanisms differ between pathways, post synaptic calcium elevation is critical to bidirectional plasticity in both (Shen et al., 2008;Pawlak and Kerr, 2008;Wang et al., 2006). Putatively motor-enhancing direct and putatively motor-suppressing indirect pathway MSNs (Kravitz et al., 2010) show the same pattern of activity dependent calcium elevation with turning behavior (Cui et al., 2013). Therefore, it is likely that both pathways contribute to the learning associated changes in plasticity measured in the present study, though the direction of plasticity may differ between pathways. With goal-directed learning in Shan et al., 2014, the AMPA:NMDA current ratio was potentiated in direct and depressed in indirect pathway MSNs dorsomedially. Those findings complement the dorsomedial reductions we find in LTP (contralateral) and LTD (ipsilateral) after early training, and suggest our results may reflect change in direct and indirect pathways, respectively.

Reduction in evoked plasticity after learning may indicate occlusion (Whitlock et al., 2006;Padmashri et al., 2013;Yin et al., 2009) or other metaplastic processes, *i.e.* processes influencing the extent to which plasticity can occur (Abraham, 2008). For instance it is possible that metaplasticity constrains capacity for off-task potentiation at dorsomedial synapses that were not recently potentiated; such off-task damping could permit a fine pattern of task-relevant LTP to be comparatively enhanced. On the other

hand if striatal LTP and LTD are reciprocally regulated, as explained by the BCM plasticity theory (Cooper and Bear, 2012), then reduction in evoked plasticity is likely to be occlusion, *i.e.* the saturation of plasticity in either direction. This was the framework for inferring that enhanced striatal LTD *ex vivo* indicates a history of LTP during learning (Lin, 2010). Alternatively, learning may regulate bidirectional plasticity forms independently rather than reciprocally within the striatum. We were able to distinguish these possibilities using 20Hz to induce LTD together with theta-burst stimulation to induce LTP (Hawes et al., 2013). In dorsomedial striatum we find reduced LTP without concomitant increase in LTD early in training, as well as reduced LTD without concomitant increase in LTP late in training. This refutes a reciprocal relationship between LTP and LTD. Whether reduced plasticity indicates recent occlusion or other metaplastic processes remains unclear, but our results establish that learning modulates both striatal LTP and LTD, and that these are modulated independently.

Learning and plasticity can be supported not only by synaptic change, but also by modified intrinsic excitability (Zhang and Linden, 2003;Frick and Johnston, 2005;Sehgal et al., 2013;Rogerson et al., 2014). Our whole-cell measures collaboratively show dorsomedial MSNs to be more excitable than dorsolateral MSNs early in training, specifically within the contralateral hemisphere. Altered intrinsic excitability has been causally linked to synaptic plasticity *in vivo* (Epsztein et al., 2011;Lee et al., 2012). Thus in the contralateral hemisphere, where a neural pattern driving the rewarded turn is expected to emerge, greater excitability may reflect greater capacity for information encoding dorsomedially with early training.

Excitability adjustments linked to learning are often accomplished through potassium channel regulation (Alkon, 1979;Disterhoft and Oh, 2006). The reduced LTP we report in early-trained rats may be caused by a period of elevated fast KA-type potassium channel activity, given that blocking KA currents enhances hippocampal LTP, and that these channels are transiently up regulated in the striatum with learning (Truchet et al., 2012). The slow KA current does not appear to contribute to striatal learning, as latency to the first action potential was unchanged. The curtailed LTD persistence we see with late training may be linked to enhanced SK-type potassium channel activity, as blocking these channels converts transient depression to LTD (Hopf et al., 2010). In rigidly habitual animals, blocking SK restores both goal-oriented behavior and LTD (Nazzaro et al., 2012). Aligning identified currents modulating MSN excitability with their influence on learning behavior is an important next step.

Neuronal morphology can influence excitability and plasticity by altering electrotonus and synapse distribution. In contrast to regional MSN hypertrophy which follows chronic stress (Dias-Ferreira et al., 2009), we find reduced dendritic complexity after training regardless of region. Similar dendritic reduction is reversibly induced in adult rodents by manipulating dopamine receptor or KIR activity (Cazorla et al., 2012). Thus ion channel modifications with learning potentially unite our electrophysiological and morphological findings. Both increased spine density and spine loss have been observed with learning and LTP in the neocortex and hippocampus (Knott and Holtmaat, 2008). New spine growth suggests new information pathways (Yang et al., 2014;Kuhlman et al., 2014), whereas spine loss potentially enhances signal to noise ratio

(Sanders et al., 2012;Lai et al., 2012). While we find MSN spine density unchanged, loss of dendritic length after training likely accompanies a reduction in synapses. Thus, dendritic pruning may enhance signal to noise ratios in the striatum.

This study gives novel insight into dorsal striatal changes enabling T-maze learning in the context of the classic concept of early dorsomedial and late dorsolateral engagement. Initially, task-oriented dorsomedial activity strengthens the rewarded turn (engaging contralateral LTP). With late training, specific task-handling shifts from dorsomedial to dorsolateral regions, which may now suppress unrewarded turns (engaging ipsilateral LTD), enforcing faithful execution of a well-learned turn. During this stage the dorsomedial striatum appears to busy itself in a non-turn relative manner: engaging LTD bilaterally may suppress recently relevant as well as distracting new information soon after a novel task is learned (Ragozzino, 2007;Ragozzino, 2003). Future studies can use this interaction between behavior and plasticity to identify the striatal adaptations responsible for various cognitive and locomotor aspects of skill learning.

4. CORTICOSTRIATAL LTP IS MODULATED BY PATHWAY-SPECIFIC CO-RELEASE OF OPIOIDS

Abstract

Synaptic plasticity adjusts behavior adaptively in the case of skill learning, or maladaptively in the case of addiction. Just as dopamine plays a critical role in synaptic plasticity underlying normal skill learning and addiction, endogenous and exogenous opiates modulate learning and addiction related striatal plasticity. Though the effect of opioid receptors on LTD has been characterized, their effect on LTP remains unknown. This study investigates the effect of opioid neuropeptides co-released by GABAergic MSNs on corticostriatal LTP. We optogenetically facilitate endogenous co-release from direct pathway (D1) MSNs in brain slice while recording change in the synaptic response of single MSN to theta-burst stimulation of cortical afferents. First, we demonstrate that theta-burst stimulation evokes corticostriatal LTP using the whole cell patch recording technique. Subsequently we show that optical activation of D1-MSNs during induction impairs LTP. We hypothesized that the opioid neuropeptide dynorphin, co-released exclusively by D1-MSNs, is responsible for this reduced TBS LTP. Dynorphin-activated kappa opioid receptors reside presynaptically on dopaminergic afferents and are capable of negatively regulating dopamine release. We demonstrate full rescue of LTP induced with optical activation of D1-MSNs by bath application of kappa opioid receptor

antagonist. Our findings illustrate a physiological phenomenon whereby heightened D1-MSN activity can regulate corticostriatal plasticity; the additional experiments described at the conclusion of this chapter will ultimately establish whether this indeed functions through regulation of dopamine availability. Our findings have important implications for learning in addictive states marked by elevated direct pathway activation.

Introduction

Synaptic plasticity of striatal neurons dynamically adjusts behavior adaptively in the case of skill learning, or maladaptively in the case of addiction. Dorsal striatum is critical for instrumental learning (Yin et al., 2005;Graybiel, 1995), motor skill development (Yin et al., 2009), cued action-selection (Packard and Teather, 1997), and habit formation (Yin et al., 2006), including habitual aspects of drug seeking. Synaptic plasticity in dorsal striatum may allow experience (cortical inputs paired with a dopamine reward signal) to selectively enhance critical action-outcome associations, resulting in learning to identify and perform the action yielding the optimal outcome. In support of this concept, changes in both LTP and long term depression (LTD) of corticostriatal synapses are observed *ex vivo* after learning a motor skill (Yin et al., 2009) or rewarded contingency (Hawes et al., 2015). Addictive drug use also leads to molecular adaptations in dorsal striatum characteristic of synaptic plasticity (Shen et al., 2011). Maladaptive plasticity leading to addiction results from drug-altered dopamine and opioid receptor activation, which natively shape corticostriatal plasticity.

Corticostriatal plasticity engages two populations of GABAergic projection neurons which are intermixed throughout the striatum. D1- and D2-MSNs, both of which receive glutamatergic and dopaminergic afferents, also receive axon collateral afferents from the mixed population of neighboring MSNs. In addition to GABA, MSNs co-release opioid neuropeptides, and the identity of co-released neuropeptide differs between D1- and D2-MSNs. Though both populations exhibit both LTP and LTD (Shen et al., 2008;Fino et al., 2010), they may be differentially activated to control skill learning (Shan et al., 2014), or in response to drugs of abuse. For instance, challenge with cocaine or heroin engages LTP-critical PKA and PKC pathways preferentially in D1-MSNs (Shen et al., 2011;Xie et al., 2010;Tropea et al., 2008;Pascoli et al., 2012), and drug sensitization-related LTP is restricted to D1-MSNs (Pascoli et al., 2012). If the direct pathway is more active in addictive states, then enhanced co-release from D1-MSNs may uniquely influence plasticity subsequent to drug-use.

D1-MSNs co-release the neuropeptide substance P and also the opioid dynorphin. Substance P binds to NK1 receptors located on glutamatergic afferents. As a result of synchronous burst firing in MSNs, substance P facilitates glutamate release preferentially on to D2-MSNs (Blomeley and Bracci, 2008;Jakab et al., 1996;Blomeley et al., 2009). Therefore, D1-MSN co-release may selectively facilitate glutamate transmission and promote LTP in D2-MSNs.

Dynorphin, which selectively binds to the kappa opioid receptor (KOR), has a very different effect. Kappa opioid receptors are positioned to regulate pre-synaptic release at various points in the striatal network, but in contrast to NK1 receptors, KOR

reduce vesicle release (You et al., 1999). KOR-mediated LTD due to reduced glutamatergic release is absent in the dorsomedial striatum where we focus (Atwood et al., 2014). However KOR are also located on dopaminergic afferents where they reduce vesicle release and hasten the re-uptake of dopamine (Kivell et al., 2014a). Because dopamine is required for LTP at D1-MSNs and potentially at D2-MSNs (Pawlak and Kerr, 2008;Shen et al., 2008), this raises the possibility that D1-MSN co-release may impair dorsomedial corticostriatal LTP by reducing dopamine transmission.

D2-MSNs co-release the opioid enkephalin, which binds to Delta and Mu opioid receptors located on glutamatergic afferents. These receptors reduce vesicle release, and their activation by bath applied agonist is capable of producing long-term depression of glutamatergic transmission onto MSNs in both medial and lateral striatal regions (Atwood et al., 2014). Therefore the co-release of enkephalin from D2-MSNs may impair corticostriatal LTP measured dorsomedially.

In summary, opioid receptor activation produces LTD in corticostriatal terminals (Atwood et al., 2014) but its role in LTP has not been investigated. Therefore, we evaluate modulation of TBS LTP by MSN cell class specific co-release using Cre-dependent expression of channel rhodopsin to elevate endogenous co-release during plasticity induction. We indeed find that elevated D1-MSN co-release during induction engages KOR to restrict corticostriatal LTP.

Materials and Methods

Animals

All animal handling and procedures were in accordance with the National Institutes of Health animal welfare guidelines and were approved by the George Mason University IACUC.

From MMRRC we obtained mice expressing Cre recombinase co-localized with D1-type dopamine receptor expression (stock Tg(Drd1a-cre)EY217Gsat/Mmucd), and mice expressing Cre recombinase co-localized with A2a-type adenosine receptor expression (B6.FVB (Cg)-Tg(Adora2a-cre) KG139Gsat/ Mmucd). From Jackson Laboratory, we obtained Ai32 mice containing genes for channel rhodopsin and EYFP downstream of a *loxP*-flanked STOP cassette (B6; 129S-Gt(ROSA) 26Sortm32 (CAG-COP4*H134R/ EYFP) Hze/J). In the offspring of Cre-line mice crossed with Ai35 mice the stop cassette is deleted in neurons expressing Cre recombinase, allowing expression of channel rhodopsin and EYFP in the Cre expressing neurons.

We generate experimental subjects by crossing males from either the D1Cre or the A2Acre line with female Ai35 mice. We refer to the resulting mice as D1CreChY and A2aCreChY, respectively, to note the Cre-driven expression of channel rhodopsin (Ch) and also EYFP (Y). Because the Cre-line males are heterozygotes, not all offspring will express Cre enabling expression of transgene from the homozygote Ai 35 mother. Genotyping to test for Cre and also epifluorescent detection of yellow fluorescence identifies Cre+ littermates expressing transgene. The D1Cre line Tg(Drd1a-cre)EY217Gsat/Mmucd was selected for the absence of Cre in cortex, but this line has

sparse expression within D1-MSNs. Therefore light-responsive MSNs in D1CreChY animals are identified as D1-MSNs, but the non light-responsive MSNs are a mixture of both D1- and D2-MSNs. Expression is more consistent in the A2aCreChY mice so that light-responsive MSNs are D2-MSNs, and most if not all of the non light-responsive MSNs are D1-MSNs.

Light Source

A CoolLED pE system supplies 500nm light for EYFP excitation, and 470nm light for channel rhodopsin activation. Light enters the light path of an Axioskop 2 FS plus Zeiss microscope through a collimated adaptor, and is directed at the striatal slice surrounding the patched cell, through a 40x submersion lens. Light intensity at the tissue is adjusted by an analog dial to assess action potential threshold as described below. In most light-responsive cells, light can be used to elicit action potentials, and is adjusted to a sub-threshold intensity for use in TBS. As a result, the light intensity used in experiments ranges from 10% to 100% of the maximum supplied by the CoolLED system.

Whole-cell Recordings

During whole cell recordings, a hemi-slice was transferred to a submersion recording chamber (ALA Science) gravity-perfused with oxygenated aCSF at 2.5-3 mL/min and 30-32°C containing 50µM picrotoxin (Tocris Bioscience) and 400µM L-ascorbic acid and 10µM glycine. Pipettes (resistance 4.5-6.8 MΩ) were pulled from borosilicate glass on a P-2000 puller (Sutter Instruments) and filled with a potassium based internal solution (in mM: K-gluconate 132, KCl 10, NaCl 8, HEPES 10, Mg-ATP

3.56, Na-GTP 0.38, EGTA 0.1) of pH 7.35. Cells were patched under visual guidance using IRDIC imaging (Zeiss Axioskop2 FS plus). Intracellular signals were collected in current clamp and filtered at 3 kHz using an EPC 10 amplifier and Patchmaster software (HEKA Electronik). Series resistance (8MΩ) was compensated 100%, and capacitance was not compensated.

EPSPs were evoked by stimulating white matter overlaying dorsomedial striatum with a tungsten bipolar electrode (diameter 0.005'' bare, 0.007'' Teflon-coated, A-M Systems) at an intensity producing a 5-15mV EPSP. EPSPs were sampled at 0.03 Hz pre- and post-induction. Current-voltage (IV) and current-frequency (IF) curves were recorded from each cell using 400ms current injection to characterize the recorded cell. Cells were determined to be MSNs by their low resting membrane potential (near -80mV), rounded AHPs, and long latency to first action potential. Cells were determined to be FSIs by their deep AHPs and narrow action potential widths. Firing threshold was determined from a series of 70 ms depolarizations produced with either current injection or optical activation. The lowest current or light intensity eliciting an action potential was considered threshold; depolarization was adjusted to be just sub-threshold for use in plasticity induction.

Plasticity Induction

Plasticity induction was accomplished by adapting the protocol described in Hawes et al. 2013 to include either somatic current injection or optical depolarization. LTP was induced by theta-burst stimulation (TBS) consisting of ten trains, each train consisting of ten bursts at 10.5 Hz (theta), and each burst consisting of four stimuli at 50

Hz, with trains spaced 15 seconds apart. 70ms depolarizing currents (beginning 10ms before the first afferent stimulation and spanning each burst) were applied to patched MSNs during TBS either via somatic current injection or else optical activation of channel rhodopsin. The amount of depolarization was adjusted to be just sub-threshold for action potential generation without afferent stimulation, as determined by a series of 70 ms somatic or optical depolarizations. To test the effect of light-induced population activation on non light-responsive cells, somatic depolarization to the patched MSN and optical activation of the population were simultaneously applied during theta burst stimulation. To test light-free plasticity in light-responsive cells, somatic depolarization is used alone.

Data Analysis

Raw data was analyzed in Python v2.7. EPSP peak amplitude and ascending slope (between 20%-80% of peak height) were extracted automatically from the raw data surrounding each test pulse. During automated amplitude extraction, traces from each experiment were graphically displayed for review by eye, guarding against errors in data extraction. Statistical analysis was performed on the EPSP amplitude and slope normalized to the pre-induction baseline. Statistical analysis was carried out in SAS (v9.3, SAS Institute). In some cases compound EPSPs were evoked; in all cases only the primary (first) EPSP following afferent stimulation was analyzed to determine plasticity. Plasticity is assessed by comparing EPSP peak amplitude or slope averaged over a 5 minute baseline (preceding induction) to the same measure averaged over a 15-20 minute

window post induction. Only cells surviving a minimum of 20 minutes post induction are analyzed. Figures were made using IGOR (v6.1.2.1). Error bars illustrate SEM.

Results

To evaluate whether LTP was modulated by MSN co-release, we induced LTP onto MSNs by pairing pre-synaptic TBS with post-synaptic depolarization in whole-cell recordings from D1CreChY or A2aCreChY expressing mice and litter mate controls. MSNs were depolarized during induction either by somatic current injection or by light activation of channel rhodopsin. Light activation produces depolarization not only in the recorded neuron, but also in the surrounding population of light sensitive neurons, resulting in enhanced release of GABA and neuropeptides. All recordings were made in the presence of 50μM picrotoxin to eliminate the effect of $GABA_A$ receptors.

TBS LTP induced in patched MSNs differs by transgenic mouse line

TBS paired with somatic current injection (without light) induces significant LTP in MSNs in D1CreChY line mice. This LTP is analyzed 15-20minutes post induction as described in Methods (Fig1A black trace, 125±5%, ttest vs. 100% DF=20 P<0.0001). Plasticity results show no relationship to baseline signal amplitude (GLM, $F_{1,20}$=0.01 P=0.9231) which averaged 11±0.7mV. Both Cre+ mice which express the transgenic channel rhodopsin and fluorophore as well as their Cre- littermates show LTP (Cre+ 123±9%, ttest vs. 100% DF=8 P=0.0317; Cre- 126±6%, ttest vs 100% DF=11 P=0.0006).

Because of sparse expression of the transgene, light-insensitive neurons are a mixed population of D1- and D2-MSNs, though D2-MSNs may be over-represented.

Change in the primary, synaptically evoked EPSP was analyzed to determine plasticity. This primary EPSP appears as a single, evoked event in approximately half of our whole-cell recordings. The other half demonstrate compound EPSPs, i.e. secondary events follow the evoked and analyzed primary EPSP. During baseline recordings, primary EPSP amplitude did not differ between cells demonstrating single or compound EPSPs (compound 12±0.8mV vs single 10±0.9mV; ttest DF=19 P=0.0875). As stated above, no relationship exists between baseline EPSP amplitude and plasticity results. Similarly, no relationship exists between the baseline frequency of compound EPSPs the amount of LTP 15-20 minutes post induction (GLM, $F_{1,20}$=3.25 P=0.0875). This suggests that the success of TBS to evoke LTP does not depend on whether a compound signal is being recorded. In the absence of light, signal size 15-20 minutes post induction does not differ between cells demonstrating compound or single EPSPs, though signal growth is smaller in the case of single EPSPs (Fig1A, compound in green 132±7% vs single in pink 120±6%; ttest DF=19 P=0.2269).

Figure1.

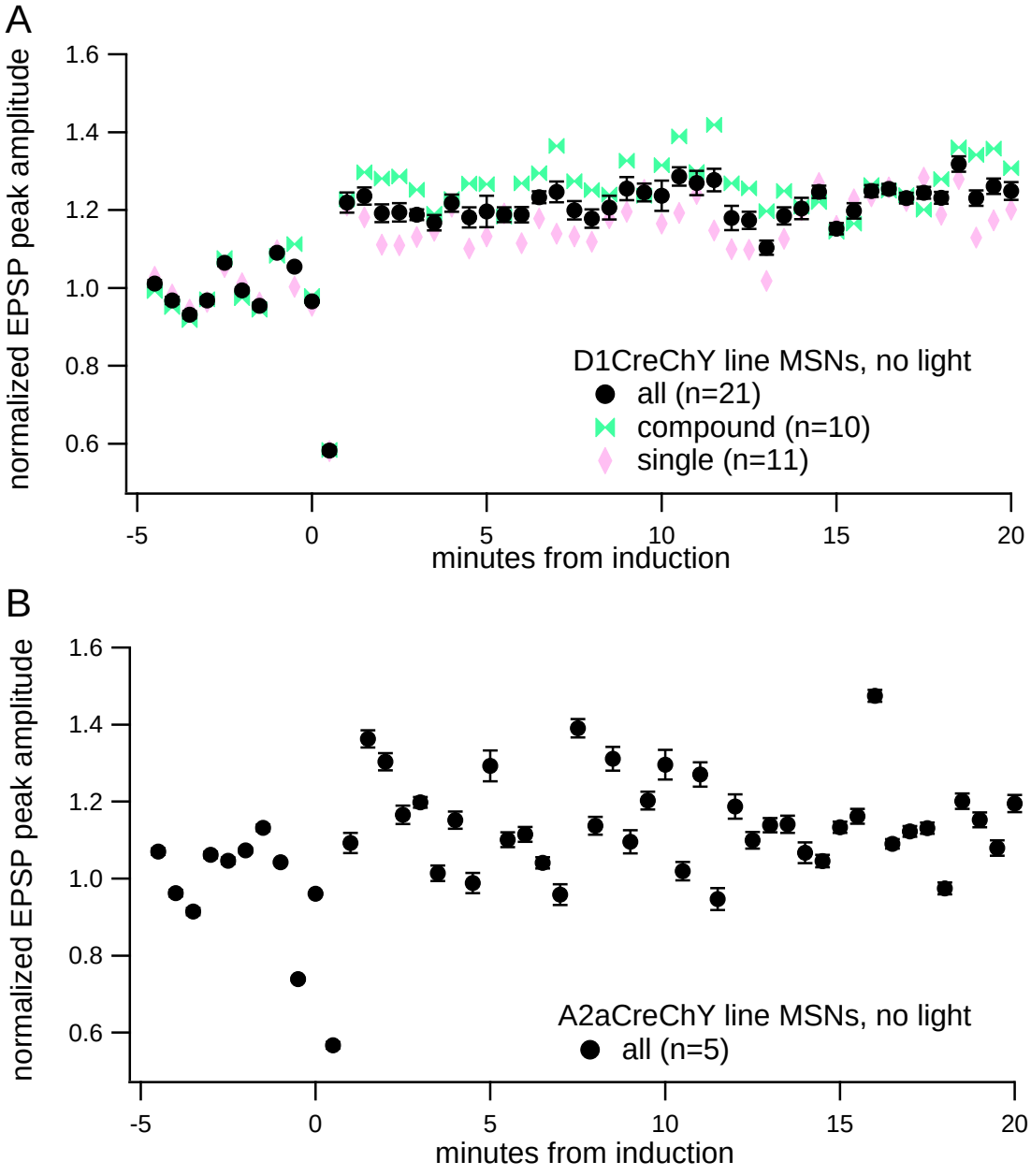

Figure 1. TBS LTP can be achieved whole-cell. **A.** MSNs patched in D1CreChY line mice express TBS LTP which is slightly more pronounced in cells demonstrating compound (subgroup average in green markers) as opposed to single EPSPs (subgroup average in pink markers). **B.** MSNs patched in A2aCreChY line mice do not potentiate.

In contrast to the D1CreChY line, preliminary recordings in A2aCreChY mice fail to demonstrate LTP in response to TBS (without light). The result of TBS 15-20 minutes post induction (116±12%, ttest vs. 100% DF=4 P=0.2627) is shown in Fig1B for A2aCreChY line MSNs. Also of note in this mouse line, we observed an undesirable amount of cortical expression of Cre-dependent transgenes. Cortical expression of channel rhodopsin provides an avenue for optical activation during TBS to influence induction in an unpredictable way, outside of the intended enhancement of D2-MSN co-release. Both because TBS LTP is not evident, and because the expression pattern of transgene is not restricted to the striatum, we did not pursue experiments in this mouse line.

Optical D1-MSN activation during induction impairs LTP

In D1CreChY+ mice, blue light is used to activate channel rhodopsin and elevate D1-MSN activity during delivery of TBS. In the absence of blue light, D1CreChY line MSNs potentiate 125±5% 15-120 minutes post induction (as reported above). However when TBS coincides with optogenetic D1-MSN activation, LTP is impaired (absent) 15-20 minutes post induction (Fig2A, 113±8%, ttest vs. 100% DF=12 P=0.1322). This result is not different between light-responsive (LR) and non responsive (non) MSN populations (Fig2A LR in gold 117±10% n=9 vs. non in gray 104±16% n=4, ttest DF=11 P=0.4917). However, we find that LTP in cells demonstrating single EPSPs in baseline recordings may be more susceptible to impairment by light than are cells demonstrating compound EPSPs (Fig2B, compound in green 127±14% n=5 vs single in

pink 104±9% n=8; ttest DF=11 P=0.1883). Our results indicate that elevated activation of the D1-MSN population during TBS impairs LTP, and suggest that susceptibility to this impairment co-varies with the factor(s) distinguishing compound or single EPSPs MSNs. Additional experiments described at the end of this chapter will investigate the difference between these groups.

Figure 2.

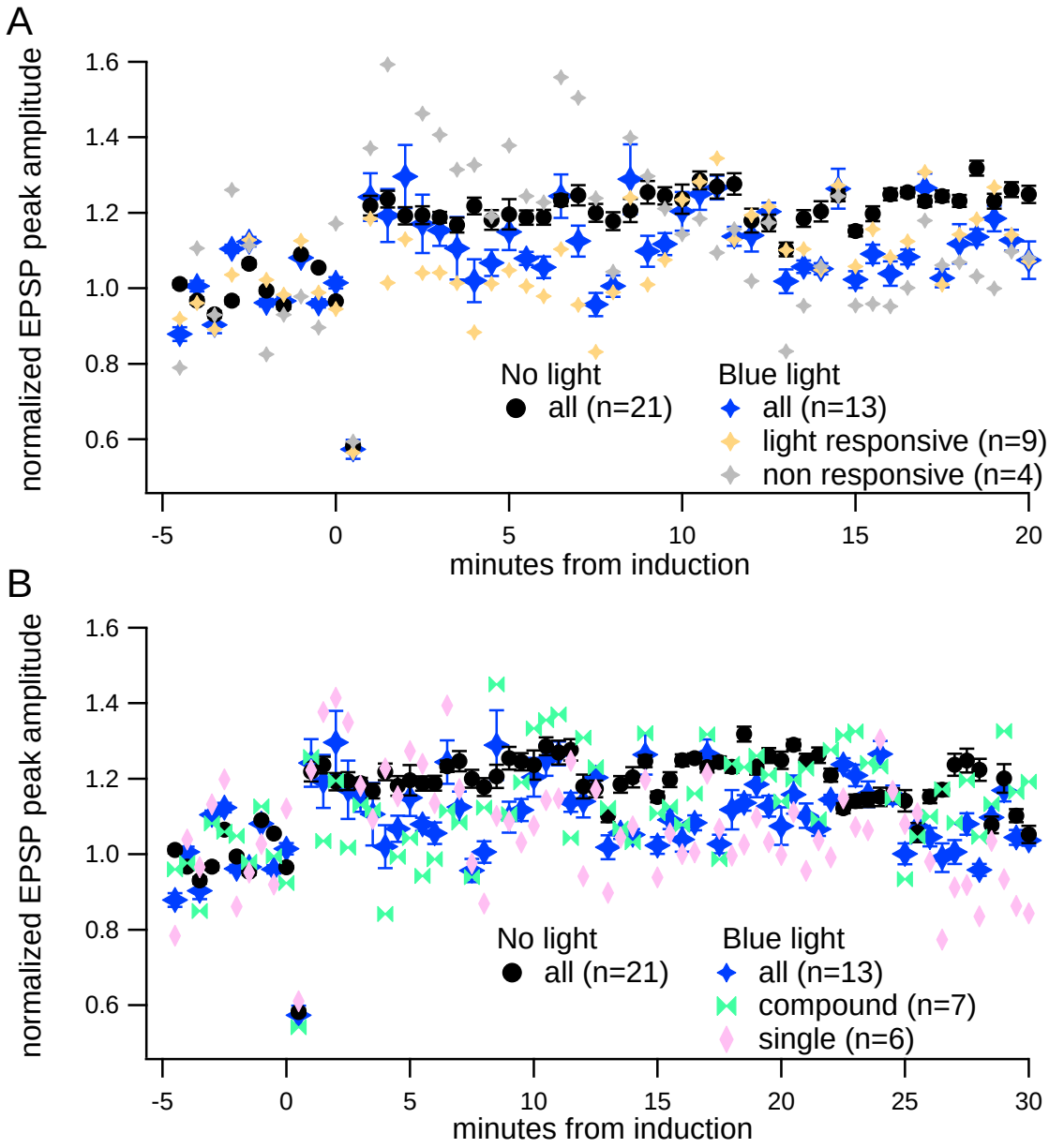

Figure 2. Optical impairment of LTP by optically activating D1-MSNs during TBS. **A.** Blue light impairs LTP; this effect does not differ between light-responsive (subgroup average in gold) and non light-responsive MSNs (subgroup average in gray). **B.** MSNs demonstrating single EPSPs are more susceptible to optical LTP impairment (subgroup average in pink) than are MSNs with compound EPSPs during baseline (subgroup average in green).

LTP is rescued by KOR antagonist during optical D1-MSN activation

The impairment of LTP through optogenetic activation of D1-MSNs does not result from elevated activation of $GABA_A$ receptors, because all experiments are performed in the presence of 50μm picrotoxin. MSN collateral release has been shown to mildly reduce glutamate release onto fellow MSNs by engaging $GABA_B$ (Logie et al., 2013), and a potential role for $GABA_B$ in impairing LTP will be tested, as described at the end of this chapter. Here, we tested whether the impairment of TBS induced LTP is attributable to opioid receptor activation by D1-MSN co-release. Because dynorphin co-release may reduce dopamine transmission via presynaptic KOR on dopamine afferents, we tested the hypothesis that optogenetically enhanced co-release of dynorphin impairs LTP using the selective KOR antagonist NorBNI.

Results show that 1μM of bath-applied NorBNI rescues TBS LTP from impairment by optical activation of D1-MSNs. LTP induced with blue light activation of D1-MSNs is rescued in the presence of NorBNI, analyzed 15-20 minutes post-induction (Fig3A in red, 142±11%, ttest vs. 100% DF=9 P=0.0052). NorBNI rescue of LTP is pronounced in the single EPSP group; LTP in the compound EPSP group remains intact (Fig3B, compound in green 136±12% vs single in pink 149±23%; colored lines facilitate comparison to analogous, drug-free groups).

Figure 3.

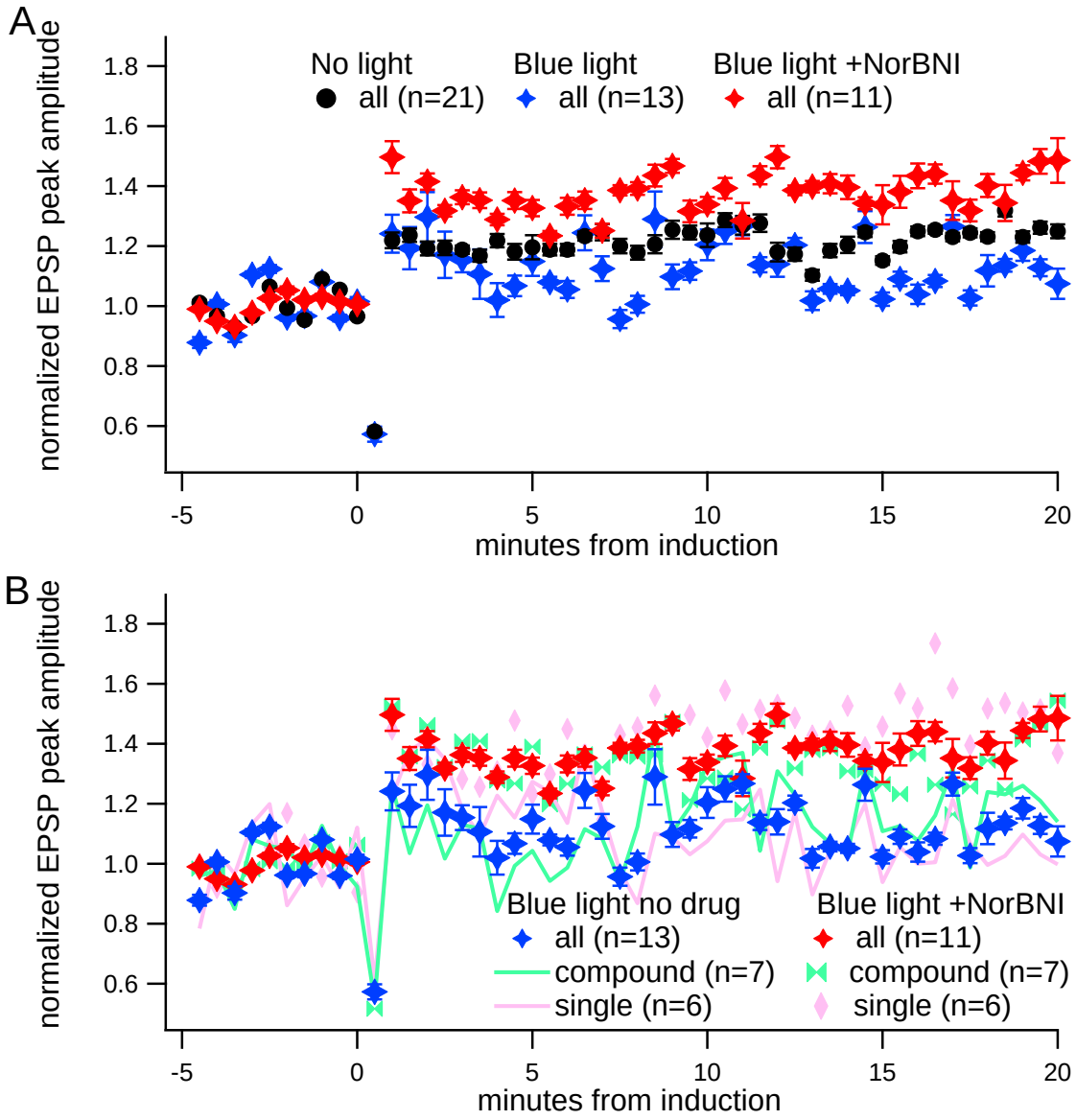

Figure 3. Optical impairment of LTP is rescued by Kappa opioid receptor antagonist. **A.** KOR antagonist NorBNI (1μM) rescues LTP in the present of blue light. **B.** Rescue is most pronounced among MSNs with single EPSPs (single EPSP subgroups in pink, compound EPSP subgroups in green; markers indicate average with drug, lines indicates averages without drug).

Presently, our findings support the hypothesis that endogenous D1-MSN co-release restricts LTP by activating KOR. However it is possible that blocking KOR facilitates LTP by reversing the optically-engaged mechanism of impairment, or it may promote LTP by another route. The first experiment done in the presence of NorBNI without optical activation indeed shows large LTP (173%) 15-20 minutes after induction, and adding to this group will test for nonspecific influence of NorBNI on LTP. Additional experiments described at the end of this chapter will establish whether optical activation of D1-MSNs during TBS reduces dopamine, and whether KOR rescues LTP through the regulation of dopamine.

Robust theta burst LTP is induced in FSIs

Most research on striatal synaptic plasticity has focused on the MSN; however, fast-spiking interneurons (FSI) exhibit STDP (Fino and Venance, 2011), and the LTP observed with field recordings using TBS may be caused by LTP onto both MSNs and interneurons. FSIs exert strong GABAergic control over MSN activity. Understanding corticostriatal plasticity in interneurons is important as these supply network level balance between MSN classes (Damodaran et al., 2014), and may regulate how closely the striatum follows the cortex (Damodaran et al., 2015).

TBS induced significant LTP in each of four patched FSIs 15-20 minutes post-induction, all in the D1CreChY mouse line (Fig4). Two experiments done without light to activate channel rhodopsin showed large LTP (one without drug 154%, one with NorBNI142% 15-20min post induction). The other two FSI recordings were done in the

presence of blue light driving channel rhodopsin and opioid co-release from D1-MSN. These showed similarly large LTP (one non light-responsive 119% and one light-responsive 139%15-20min post induction). The observation that one FSI was light-responsive and expressed LTP indistinguishable from the other patched FSIs suggests expression of transgenic channel rhodopsin and EYFP do not influence plasticity in FSIs. In conclusion, this important interneuron group expresses robust LTP in response to theta-burst stimulation. In contrast to MSNs, LTP in FSI appears to be insensitive to optically driven co-release from D1-MSNs.

Figure 4.

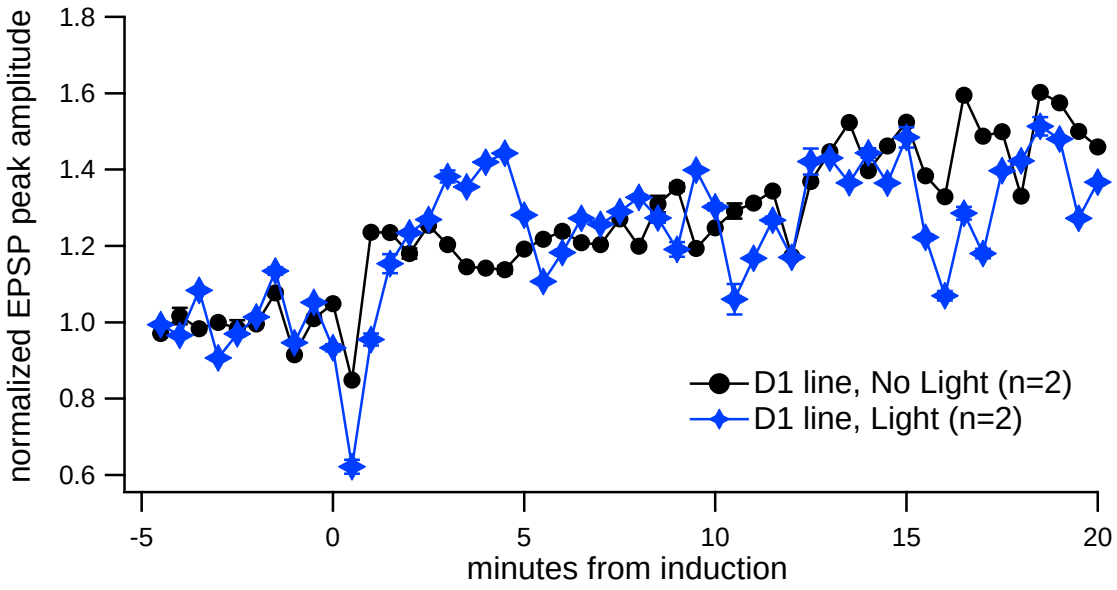

Figure 4. TBS LTP is seen in fast-spiking interneurons without optical impairment.

Analysis of MSN plasticity using EPSP slope

An alternative measure of EPSP change is the rising slope between 20% and 80% of the peak. We analyzed EPSP slope in addition to peak amplitudes, and find that slope analysis reflects the same plasticity results as does EPSP peak analysis, but with larger variance. Figure 5 illustrates the same comparison shown in figure 3A (D1CreChY line LTP in the presence and absence of light and drug) using EPSP slope rather than peak. Slope increased with TBS in the absence (137±6%, ttest vs. 100% DF=20 P<0.0001) but not in the presence of light (121±13%, ttest vs. 100% DF=12 P=0.1293). Finally NorBNI increases LTP measured by EPSP slope in the presence of light (131±11%, ttest vs. 100% DF=9 P=0.0159).

Figure 5.

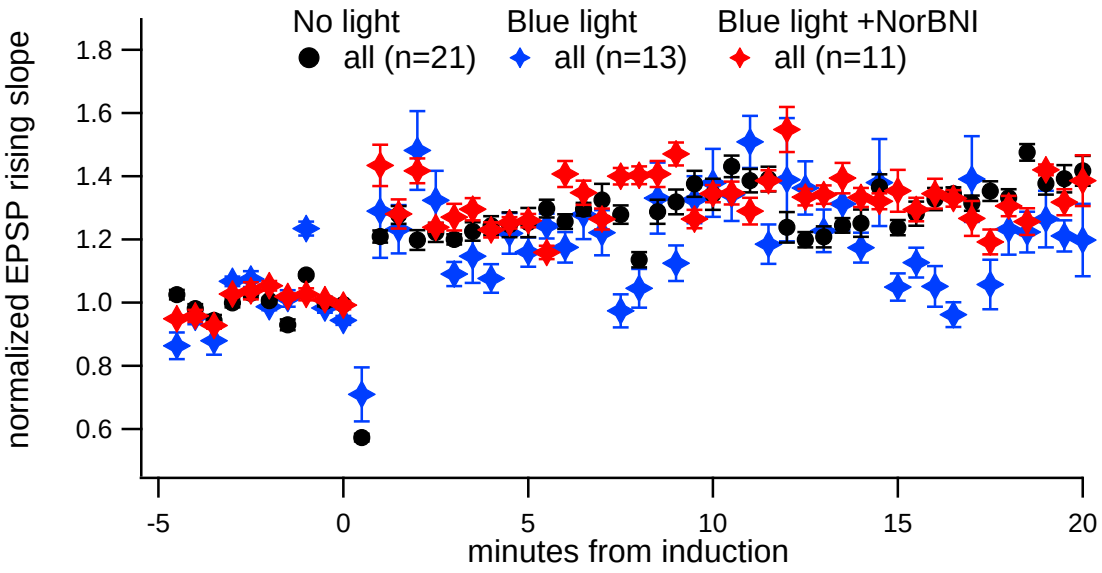

Figure 5. EPSP rising slope is a more variable measure than EPSP peak amplitude in our data. Figure 5 uses EPSP slope to make the same group comparison presented in figure 3A using EPSP peak.

Similarly, slope analysis reflects the same relationship between single and compound EPSPs and plasticity results. In the absence of light both compound and single EPSP signals potentiate without difference (compound 142±10% vs single 133±8%; ttest DF=19 P=0.5135). LTP in compound EPSPs is resistant and in single EPSPs is susceptible to impairment by blue light (compound 150±21% vs single 103±14%). Again, NorBNI rescues LTP in the single EPSP group and LTP in the compound EPSP group remains intact (compound 133±12%; single 128±23%).

Discussion

This study demonstrates modulation of corticostriatal LTP by endogenous, intra-co-release of the opioid neuropeptide dynorphin. In particular we show that elevated activity in the D1-MSN population is capable of inhibiting corticostriatal plasticity through actions at kappa opioid receptors (KOR). Targeted manipulation of KOR has been shown to modulate anxiety, memory, and drug-seeking behavior (Kivell et al., 2014b;Crowley and Kash, 2015;Menard et al., 2013). Thus our findings have important implications for learning and particularly for plasticity regulation in addictive states marked by elevated D1-MSN activation (Shen et al., 2011).

Difference in plasticity between MSN classes has been demonstrated (Mathur et al., 2013;Bateup et al., 2010;Pascoli et al., 2012;Valjent et al., 2010;Centonze et al., 2001;Kreitzer and Malenka, 2007;Yin et al., 2009). Others find no difference in plasticity between MSNs (Wang et al., 2006;Bagetta et al., 2011;Pawlak and Kerr, 2008). During the development of an effective theta-burst LTP induction protocol using field recordings it was unclear whether one or both MSN populations expressed the recorded LTP (Hawes et al., 2013). In this work we have shown that the same theta-burst stimulation (TBS) can be used to study dorsomedial corticostriatal LTP recorded in whole-cell current clamp mode. Given that potentiation is regularly observed in unidentified D1-MSNs in the D1CreChY mouse line (Fig1A), our whole-cell plasticity data suggest that both D1-MSNs and D2-MSNs readily potentiate in response to theta-burst stimulation. This will be further tested through experiments described at the end of this chapter.

However our MSNs are divided between those demonstrating single or compound EPSPs, and these groups show different susceptibility to NorBNI-mediated rescue LTP. It is possible that, while both MSN classes express LTP in response to theta-burst stimulation, this plasticity is more susceptible to regulation by D1-MSN co-release in one versus the other cell class. Alternatively, some MSNs may have more convergent or recurrent glutamatergic connections due to their position in the slice, and may demonstrate compound EPSPs as a result. The same factors creating compound EPSPs may promote greater dopamine release near these cells during TBS. Increased dopamine could overcome a KOR-mediated reduction in LTP. Difference in these MSN groups will be investigated as described at the end of this chapter.

The reduction in LTP caused by optically enhancing D1-MSN activity during TBS (Fig3A) is more likely to be caused by co-release of dynorphin than release of GABA because $GABA_A$ receptors were blocked with picrotoxin. D1-MSN co-release of dynorphin might be responsible for reduced LTP due to its capacity to restrict release of dopamine from nigrostriatal afferents, and corticostriatal LTP in either MSN population is known to rely on dopamine (Shen et al., 2008;Pawlak and Kerr, 2008). Dynorphin is the endogenous ligand at KOR, and these have been shown to reduce intra-striatal dopamine release (You et al., 1999). KOR are a fascinating plasticity modulator as they are synthesized in midbrain dopaminergic nuclei, and presynaptically situated to control dopamine release from SNc afferents in dorsal striatum (Bruijnzeel, 2009). Here the G_i-coupled receptor modulates both N-type Ca channels and inward-rectifying potassium channels to inhibit vesicle release (Schlosser et al., 1995), and can rapidly enhance

dopamine re-uptake through influencing dopamine transporter kinetics (Kivell et al., 2014a). Our experiments using KOR antagonist NorBNI confirm that optical-impairment of TBS LTP indeed operates through KOR. Experiments described at the end of this chapter will establish whether the influence of KOR on LTP indeed works through the control of intra-striatal dopamine release.

Because light activation of channel rhodopsin has been employed in mostly D1CreChY+ light-responsive cells so far, it may be that the light effect is due to failure of D1-MSNs to potentiate. LTP rescue with NorBNI argues against this since LTP is rescued in both light-responsive and non light-responsive MSNs. Nonetheless we will confirm that both MSN populations are capable of expressing LTP through the addition of light-free TBS experiments in identified D1-MSNs (light-responsive) in the D1CreChY mouse line, and also by identifying non light-responsive neurons using histochemical techniques as discussed at the end of this chapter.

Caveats in our ability to target expression of channel rhodopsin should be considered while interpreting results. Mouse lines used to target alternate MSN classes express Cre in cells where dopamine D1-type receptor is expressed. However in dorsal striatum, D1-type dopamine receptors are also found on fast-spiking interneurons (FSIs). Consequently, mice expressing channel rhodopsin in D1-MSNs also expressed channel rhodopsin in a subset of GABAergic FSIs; and in fact a minority of the FSI patched in this study was clearly light-responsive. Optical activation of FSIs is not likely to influence experiments because picrotoxin is present in all experiments, blocking $GABA_A$ receptors. However it is possible that activation of $GABA_B$ receptors via co-released

GABA may modulate TBS LTP, and this will be tested as described at the end of this chapter.

An additional feature of expression in our D1Cre mouse line is the curiously sparse expression among a subset of D1-MSNs (and FSI). On the one hand, this indicates that KOR-mediated LTP modulation we report is strong enough to be observed even when only a subset of D1-MSNs releases extra dynorphin. In fact elevating co-release from a sparse subset in this way may be more physiological than activating all MSNs because, *in vivo,* MSNs have low firing rates and low population coherence (Barnes et al., 2005). Secondly, it has been suggested that the sparse Cre expression pattern in this mouse line may coincide with striosome compartments (personal communication). Striosomes are small, scattered striatal subregions which have distinct neuromodulator densities, denser limbic afferents, and a theoretical role as a reward-weighing "decider" compartment within striatum (Gerfen, 1992;Miura et al., 2008). Importantly acetylcholine, which is densest in striosomes, activates nicotinic receptors located on dopaminergic afferents to enhance intra-striatal dopamine release (Threlfell et al., 2012). Dynorphin-driven KOR actions function in opposition to acetylcholine driven nicotinic dopamine release (Gauchy et al., 1991), and it is possible that these processes are more pronounced within the striosomes to balance one another. We will perform histochemical labelling to look for overlap in EYFP and striosomal markers, such as Mu opioid receptors or acetylcholinesterase, as described at the end of this chapter.

There are interesting implications for the robust TBS LTP we find in FSIs (Fig4). The FSIs are an important modulatory interneuron class within dorsal striatum. These

interneurons comprise a GABAergic network associated through gap junctions, and this network serves to desynchronize cortical influence (Damodaran et al., 2015). Our data indicate that FSIs potentiate more strongly than MSNs, which may be critical to prevent pathological cortical coherence. In other words, this feature may enable FSIs to "out-shout" the MSNs, even after corticostriatal LTP. The FSI network also helps to balance D1- and D2-MSN activity (Damodaran et al., 2014). Our data suggest that, while KOR reduce LTP in MSNs, LTP in FSIs is robust to the actions of KOR. This could be useful to preserve balance when LTP is differentially expressed between MSN populations, as occurs with addictive drug challenge (Xie et al., 2010;Tropea et al., 2008;Pascoli et al., 2012) and relapse (Pascoli et al., 2012). In summary the large and robust FSI LTP in response to cortical TBS may provide synaptic scaling in the FSI-MSN relationship to guard against pathological coherence. Meanwhile FSI insensitivity to local modulators of MSN LTP, such as dynorphin acting at kappa opioid receptors, may permit meaningful local difference in synaptic plasticity onto MSNs.

The major finding in this work is that elevated endogenous co-release from D1-MSNs limits corticostriatal LTP. This may serve to limit additional LTP during addictive states marked by disproportionate activation of the D1-MSNs, and indeed KOR agonists have drawn interest for their potential utility in anti-addiction therapy. Our results suggest that, beyond reducing dopamine reward, the KOR may be natively engaged to prevent the plasticity underlying the development and perpetuation of addictive states. Further optical dissection of striatal plasticity circuits will yield clinical targets useful in combating

addiction, and in rebalancing plasticity in an addicted network in support of healthy learning.

Continuing and Future Experiments

Confirm that LTP is expressed in both D1- and D2-MSNs

The frequency of TBS LTP success (without light) in unidentified neurons strongly suggests that both MSN populations express LTP; however, the inability to observe LTP in either MSN population in the A2aCreChY line prevents us from extrapolating from that line. Thus, to confirm that TBS LTP occurs in both MSN populations in the D1CreChY line, biocytin will be added to the internal solution during whole-cell recordings of TBS LTP, enabling the recorded neurons to be stained and identified in subsequently fixed slices. MSN class-markers, such as Met-Enk for D2-MSNs and pre-pro-Dynorphin for MSNs, will be histochemically labeled. Co-localization of biocytin with MSN-specific markers will identify MSNs, and in this way neuronal identity can be attributed to the plasticity recorded for each cell.

In an alternative and complimentary approach, we may use intrinsic characteristics of recorded MSNs to designate D1-likely and D2-likely MSN subsets within unidentified neurons. On average, D2-MSNs are more intrinsically excitable, with smaller latencies to fire and lower rheobase than D1-MSNs. However, there is a great deal of overlap in these measures between the two populations, meaning that many MSNs demonstrate latency to fire or rheobase measures which could belong to a cell from either

group. However the cells exhibiting the upper-most 10% of these measures, and the cells exhibiting the lower-most 10% of these measures are likely to be D2-MSN and D1-MSN respectively. Comparing plasticity results between these groups should indicate whether one MSN expresses LTP and the other does not.

Investigate distinction between MSNs with single versus compound EPSPs

The above investigation into MSN class will address whether either class demonstrates predominantly single or compound EPSPs. We can also analyze the number of action potentials and mean depolarization during theta-burst stimulation to see if these are greater in MSNs with compound-EPSPs. Focal perfusion of picrotoxin at the patched MSN will permit cortical inhibition, and if this eliminates compound EPSPs then we will have identified their source. If it impairs LTP, this suggests increased cortical activation of all MSNs facilitates LTP rather than MSN populations differing.

Test for $GABA_B$ contribution to optical LTP impairment

Despite the full rescue of optically-impaired LTP by NorBNI, MSN collateral release has been shown to mildly reduce glutamate release onto fellow MSNs by engaging $GABA_B$ receptors (Bracci and Panzeri, 2006), and therefore it is possible that optical enhancement of MSN co-release impairs LTP in part through increased $GABA_B$ activation. To test this, we will administer the $GABA_B$ antagonist saclophen (or phaclophen), similar to NorBNI, to establish whether this is similarly capable of LTP rescue.

Investigate whether we are preferentially inciting co-release from striosomal MSNs

The sparse distribution of Cre expression among D1-receptor expressing cells may reflect preferential expression within the striosomal compartments. Because these compartments have distinct neuromodulatory composition and are proposed to serve specialized roles in striatal processing, it is important to recognize whether our data indicates striosome-specific plasticity regulation. We will use immunohistochemical labeling of EYFP and striosomal markers, such as Mu opioid receptors or acetylcholinesterase. Overlap in EYFP and striosome labeling would identify Cre-expressing cells as belonging to striosomal regions. This would augment the story that D1-MSN co-release impairs LTP, by attributing this role to striosomal D1-MSNs in particular within our experimental paradigm.

Establish whether KOR impairs LTP by limiting dopamine transmission

We have shown that KOR action restricts corticostriatal LTP, but KOR may be located presynaptically on glutamatergic as well as dopaminergic afferents. Reduction in the release from either afferent source may be responsible for the optical LTP impairment rescued by NorBNI, and both may contribute.

We hypothesize that co-release of Dynorphin activates KOR located on dopaminergic afferents, and reduces LTP as a result of reducing dopamine transmission. To test this hypothesis, we will apply a DA-reuptake inhibitor during experiments in which D1-MSNs are light-activated with TBS. If this rescues LTP similar to NorBNI, it

will suggest that dopamine transmission is compromised by D1-MSN co-release to impair LTP. Of course enhancing dopamine transmission may overcome various deficits to promote corticostriatal LTP. Voltammetry will provide a powerful complement to DAT inhibition. In D1CreChY mice, dopamine levels during TBS in the presence of light-activated co-release will be compared to dopamine levels during standard TBS using voltammetry. Finding dopamine reduced in the presence of light will confirm that optogenetically enhancing D1-MSN activity indeed reduces dopamine transmission with TBS.

Testing for a contribution of KOR located on glutamatergic terminals will be more difficult. This may require KOR deficient mutants in which the deficiency in receptor expression is targeted to glutamatergic neurons. Therefore this is a future investigation outside the scope of our work.

5. GENERAL IMPLICATIONS AND FUTURE DIRECTIONS

My dissertation research attempted to identify synaptic plasticity mechanisms underlying striatal dependent learning. Rather than focusing solely on LTD, I first developed a theta-burst LTP induction protocol, and then used this protocol to investigate changes in both LTP and LTD, both in dorsomedial and dorsolateral striatum after procedural learning. One feature which has proven useful in investigating the alignment between changes in plasticity and behavior is the variation in subregion engaged at various stages in learning. Another useful feature is likely to be MSN class difference: D1R-MSNs enhance movement, whereas D2R-MSNs inhibit movement; thus plasticity in these two cell classes is likely to have different effects on behavior. Regional plasticity distinctions were investigated to clarify substrates for different stages and modes of learning. Chapter 3 of this dissertation finds altered striatal plasticity relative to turn-learning which may reflect equal or unequal engagement of MSN populations to drive the rewarded turn. Turning is associated with elevated striatal activity in the hemisphere opposite (contralateral to) the turn; and interspersed MSNs belong either to the motor-enhancing "direct" or else to the motor-suppressing "indirect" pathway. Combining these motifs, learning to turn may consist of potentiation of direct pathway D1-MSNs in the contralateral hemisphere. Therefore I predict the reduced contralateral LTP early on in the dorsomedial region results from LTP predominantly among D1-MSNs. At the same

time LTP among indirect pathway D2-MSNs may underlie the slight reduction in dorsomedial LTP ipsilaterally. Similarly, I would expect to find LTP predominantly among direct pathway MSNs contralaterally and indirect pathway MSNs ipsilaterally in late-trained animals if recording dorsolaterally, as these changes theoretically support performance of the learned turn. I predict predominant depression among direct, "go" pathway MSNs ipsilaterally drives the learned turn in late-trained animals, resulting in our observation of reduced dorsolateral LTD. Though whole-cell TBS LTP experiments in identified neurons and after lateralized learning could be used to test these hypotheses regarding class differences in LTP, other techniques can be applied as well. For instance an immunohistochemistry approach recently demonstrated a metaplastic NMDA subunit shift presumed to facilitate plasticity contralaterally while the opposite shift coincides with reduced AMPA ipsilaterally (Kent et al., 2013).

The inability to elicit LTP dorsolaterally impeded our ability to observe changes in LTP in that region following T-maze training in Chapter 3, which may be due to a regional difference in LTP efficacy of theta-burst stimulation documented in Chapter 2. A recent publication evokes LTP dorsolaterally using 5Hz theta frequency (Park et al., 2014), which I was not able to do reliably using 10.5Hz theta bursts and indeed theta coherence dominates at a lower frequency dorsolaterally than dorsomedially as animals learn (Thorn and Graybiel, 2014). Furthermore, though I showed in Chapter 2 that D1R are required for dorsomedial LTP, the study by Park et. al. 2014 indicates that dorsolateral LTP depends on BDNF but is independent of D1-type dopamine receptor signaling. It will be important to test alternative theta-burst frequencies across regions

within the same study to definitively show a region difference in theta frequency dependence. However if the two dorsal striatal subregions indeed express LTP in response to dissimilar afferent timing, it is reasonable that LTP between regions may also depend on dissimilar effectors. Action-outcome learning served by the dorsomedial striatum relies on rewarded contingencies; thus it is natural that dorsomedial LTP would rely on dopamine which is released in response to reward. In contrast, stimulus-response learning and habitual performance of over-trained skills served by dorsolateral striatum are relatively insensitive to reward. Therefore it is logical that dorsolateral LTP would not require dopamine. If dorsolateral LTP does not require dopamine, then infusing D1-type dopamine receptor antagonist may block both LTP and reward-contingency acquisition medially without effect when infused laterally. In contrast, inhibiting BDNF receptors laterally may block LTP and disrupt motor skill learning. Mechanistic distinction between plasticity in these regions is clinically important. In addicts, dorsolateral striatal plasticity fostering outcome-insensitive habits drives drug-seeking despite desensitization to initial hedonic effects, and despite negative action-outcome contingencies (i.e. getting fired, divorced, loss of health, etc.).

Chapter 4 of this dissertation investigates another receptor distinguishing dorsomedial and dorsolateral plasticity, and which is also a target of interest for the design of anti-addiction therapies: the Kappa opioid receptor (KOR). A recent publication shows KOR agonist leads to LTD through reduced presynaptic glutamate release in dorsolateral but not dorsomedial striatum (Atwood et al., 2014), suggesting that KOR expression at glutamatergic afferents is higher dorsolaterally. This can be tested with

immunohistochemistry to compare co-localization of KOR in either region with markers for glutamatergic (Vglut 1 and 2) or else dopaminergic (DAT) afferent terminals. KOR are also located on pre-synaptic dopamine terminals, and that expression may be higher dorsomedially. Results in Chapter 4 of this dissertation illustrate dorsomedial LTP modulation by endogenously engaged KOR, and I hypothesize that KOR reduce dorsomedial LTP by reducing dopamine release. This will be tested using voltammetry and DAT inhibition, as described at the end of Chapter 4. The presence of KOR-LTD dorsolaterally suggests that LTP might be modulated by direct pathway co-release in both the dorsomedial region, which I tested, and also in the dorsolateral region which I did not test due to reduced success of 10.5Hz theta-burst to produce LTP laterally. Given a useful dorsolateral induction method, it is likely that the dorsolateral striatum would also show KOR-mediated reduction in LTP, but I hypothesize that this would arise from pre-synaptic reduction in glutamate (Atwood et al., 2014). If this is true, then the anticipated rescue of LTP dorsomedially by DAT-blockers should fail to rescue LTP dorsolaterally, particularly if dorsolateral LTP is insensitive to dopamine.

Focusing on the dorsomedial striatum in Chapter 4, I find corticostriatal LTP impaired by the endogenous co-release of dynorphin from direct pathway MSNs. Addicted states are characterized by heightened direct pathway activation and relapse behavior is accompanied by LTP-like changes.

Following chronic opioid self-administration, acute challenge with a novel opiate does not produce the same reduction in mEPSCs in D1-MSNs as is seen both in D2-MSNs after self-administration, and in drug-naïve animals (James et al., 2013). Therefore

the *ex vivo* optogenetic direct pathway activation studied in Chapter 4 may model an addicted network, or else relapse plasticity. Given that endogenous co-release of dynorphin blocks LTP in my experiments, I hypothesize that dynorphin release may function as a negative feedback loop – dampening LTP in response to addictive drugs. Reduced KOR expression or function may be permissive for susceptibility to drug addiction or relapse; therefore differences in KOR expression may be relevant for studying differences in innate susceptibility or resilience to drug abuse.

Though this dissertation focused on synaptic plasticity, I also observed elevated intrinsic plasticity that was transient, disappearing late in training. The transient contralateral enhancement in dorsomedial MSN excitability with early-training may facilitate synaptic plasticity, or it may function to facilitate the rewarded turn execution more directly. Though the cause of the altered intrinsic excitability was not identified, prior research has suggested several potassium channels that could account for the results, and have been connected to other learning tasks. I believe that upregulated Kv4.2 channels underlie this enhanced dorsomedial intrinsic excitability in the contralateral hemisphere, and that this change contributes to the reduction in evoked LTP. These channels are transiently up regulated in the striatum with spatial learning (Truchet et al., 2012) at the same time that we find reduced evoked LTP. Overexpression of Kv4.2 not only enhances intrinsic excitability and reduces LTP in hippocampal neurons, but also leads to a reduced NR2B:A ratio at the synapse which represents a metaplastic state in which LTP is harder to evoke (Jung et al., 2008;Yashiro and Philpot, 2008). Involvement of Kv4.2 with early training may be tested using voltage clamp or immunohistochemistry,

and confirmed behaviorally. Altered Kv4.2 expression and NR2B:A alteration can be tested with quantitative immunoblots. Testing *ex vivo* plasticity, I would expect KA inhibition to facilitate LTP more in early-trained than in naïve rats, due to rescuing the hypothesized increase in KA-type currents early in training. I believe that elevated KA makes neurons more excitable but less likely to retain information through LTP, and that this improves the signal: noise ratio as only the most relevant information is acquired. If this is true, then KA-facilitation in one hemisphere just prior to T-maze training (either by drug infusion or diet-controlled transgene expression such as the tet-off system) should make it more difficult for animals to learn to turn in the direction contralateral to the KA boost, as they would have generally impaired LTP prior to presentation of the rewarded contingency. However if KA is facilitating learning by increasing cell firing to promote rewarded motor patterns, then the same treatment would assist animals in learning a turn contralateral to the KA boost.

This dissertation provides a useful LTP induction method, and with it, is able to describe several new and fascinating components to striatal learning. The most important future studies will be those which meaningfully integrate disparate components in learning (brain region, cell class, molecular effectors, and synaptic, intrinsic, and morphological plasticity) to assign a more complete neural process to emergent cognition or behavior.

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