DIFFERENCES IN LTP BETWEEN DORSOMEDIAL AND DORSOLATERAL STRIATUM: INDUCTION FREQUENCY AND D1 RECEPTORS

by

Valerie J. Lewitus

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Committee:

_____	Dr. Kim T. Blackwell, Thesis Director
_____	Dr. Karl Fryxell, Committee Member
_____	Dr. Ancha Baranova, Committee Member
_____	Dr. Iosif Vaisman, Director, School of Systems Biology
_____	Dr. Donna Fox, Associate Dean, Office of Student Affairs & Special Programs, College of Science
_____	Dr. Peggy Agouris, Dean, College of Science
Date: _____	Spring Semester 2018 George Mason University Fairfax, VA

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at George Mason University

By

Valerie J. Lewitus
Bachelor of Science
University of Mary Washington, 2014

Director: Kim T. Blackwell, Professor
Department of Bioengineering

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Fairfax, VA

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

Artificial cerebrospinal fluid.....……ACSF
Calcium-calmodulin-dependent kinase II.....CaMKII
Dorsolateral .....DL
Dorsomedial.....DM
Gamma-aminobutyric acid.....GABA
Generalized linear model.....GLM
Long-term depression .....LTD
Long-term potentiation .....LTP
Population spike.....pop spike
Protein kinase A.....PKA
Spiny projection neuron.....SPN
Theta-burst stimulation.....TBS

ABSTRACT

DIFFERENCES IN LTP BETWEEN DORSOMEDIAL AND DORSOLATERAL STRIATUM: INDUCTION FREQUENCY AND D1 RECEPTORS

Valerie J. Lewitus, M.S.

George Mason University, 2018

Thesis Director: Dr. Kim T. Blackwell

The striatum of the basal ganglia is involved in learning, such as the acquisition of precise motor control and habits. The dorsomedial (DM) striatum receives input from the association cortex and is involved in spatial learning and goal-oriented behavior, while the dorsolateral (DL) striatum receives input from the primary sensorimotor cortex and is involved in procedural learning and habit formation. Previous studies have found that neuron spiking is entrained to a higher TBS frequency in the DM than the DL striatum. In addition, previous studies have shown dopamine D1 receptors are required in the DM striatum and others show that these receptors are not required in the DL striatum. It is unclear whether the latter is due to a true regional difference or due to differing experimental protocols. To investigate regional differences in learning between these two areas, we tested whether the induction of LTP in the DM and DL striatum differs across different theta-burst stimulation (TBS) frequencies and in the presence or absence of D1

receptors. We recorded extracellular field potentials in adult male C57Bl/6 mouse brain slices in response to a theta burst stimulation of either 10.5 Hz or 5 Hz. We found that 10.5 Hz – but not 5 Hz – produces LTP dorsomedially; in contrast, 5 Hz – but not 10.5 Hz – produces similar LTP amplitudes dorsolaterally. To test for the requirement of D1 receptors, we blocked the receptors using SCH23390 in the DL striatum and found that this blocks LTP, which is similar to what has been found in the DM striatum. Additionally, in order to determine whether female mice are appropriate subjects to use in this study, we observed whether mouse sex or estrus status influences the ability to induce LTP using 10.5 Hz in the DM striatum. We found that female mice who are not in the estrus stage exhibit LTP of a similar amplitude to male mice, but female mice who are in the estrus stage do not exhibit LTP under the same stimulation protocol.

INTRODUCTION

The striatum of the basal ganglia is associated with behaviors such as the acquisition of precise motor control, instrumental learning, action-selection, and stimulus-response learning (Yin et al., 2009, Yin et al., 2005; Tecuapetla et al., 2016; Faure et al., 2005). Dysfunction in the dorsal striatum has been implicated in Parkinson's Disease, Huntington's Disease, and addiction (Mayeux, 2003; Milnerwood & Raymond, 2010; Girault, 2012). The dorsal striatum can be subdivided into two regions: the dorsomedial (DM) and dorsolateral (DL) striatum. The DM striatum is involved in spatial learning, instrumental conditioning, and goal-oriented behavior (Yin and Knowlton, 2004; Yin et al., 2005; Bradfield et al., 2013), while the DL striatum is involved in procedural learning and habit formation (Barnes et al., 2005; Yin et al., 2004), as evidenced by lesions or inactivation of these areas causing distinct behavioral deficits (Featherstone and McDonald, 2004; Chang and Gold, 2004). The reason that the two areas are responsible for differing functions and behaviors is not yet well understood.

One mechanism that may explain the difference in function between the DM and DL striatum is the anatomy of these areas. The dorsal striatum receives glutamatergic inputs from the cortex and thalamus. These inputs are received by striatal projection neurons (SPNs), which make up the majority of the neuronal population in the striatum (Bolam et al., 2000). SPNs in the DM striatum receive glutamatergic input from the

association cortex, while those in the DL striatum receive input from the primary sensorimotor cortex (McGeorge and Faull, 1989). While the difference in function between these two areas may be explained in part by their differing cortical input, not all differences may be attributed to this. Identifying the cellular differences in learning at the cellular level can give insight into mechanisms that contribute to this difference in function.

Synaptic plasticity refers to a change in synaptic strength and is one of the cellular properties of neurons that differs between brain regions. Long-term potentiation (LTP) is a prolonged increase in synaptic strength in response to a certain pattern of synaptic input and is used as a correlate of learning at the cellular level in several regions of the brain, including the striatum (Charpier and Deniau, 1997). In the technique of field recording, synaptic strengthening is observed as an increase in the voltage response of a population of neurons (pop spike) after a certain stimulation protocol. The pop spike size is the combined excitatory post-synaptic response of a population of neurons after an excitatory stimulus. The amplitude of the pop spike increases when synapses undergo LTP and decreases when synapses undergo long-term depression (LTD). Corticostriatal connections have been shown to undergo synaptic plasticity in response to certain stimulation patterns such as high-frequency stimulation (Partridge et al., 2000), spike-timing dependent plasticity protocols (Fino and Venance, 2010), and theta burst stimulation (TBS) (Hawes et al., 2013). TBS is a particularly practical protocol because theta has been found to be the naturally occurring rhythm frequency in the striatum during learning *in vivo* (Tort et al., 2008; Lepski et al., 2012). Typically, LTP is more easily induced in the DM striatum, whereas LTD is more easily induced dorsolaterally (Smith et al., 2001; Hawes et al., 2013),

but another difference between these two areas could lie in the actual stimulation frequencies required to induce LTP.

One of the reported differences in frequencies to induce LTP was found by Partridge et al. (2000) where high-frequency stimulation (100 Hz) of the DM and DL striatum resulted in LTP and LTD, respectively. In terms of TBS frequency, Hawes et al. (2013) demonstrated that LTP can be induced in the DM but not the DL striatum by delivering TBS at 10.5 Hz to the overlying white matter in the corpus callosum. The reason that this TBS frequency does not result in LTP in the DL striatum may be explained by a study that found that SPNs and interneurons in the DL striatum are naturally entrained to a lower TBS frequency than those in the DM striatum (Thorn and Graybiel, 2014). Due to this difference in natural entrained frequency, it is possible that DL corticostriatal synapses require a lower TBS frequency in order to undergo LTP. To investigate regional differences in learning between these two areas, we tested whether the induction of LTP in the DM and DL striatum differ across different TBS frequencies. It is unclear why LTP in the DM and DL striatal areas exhibit these differences, but investigation into signaling pathways may give insight into this difference.

A key signaling molecule in the striatum is dopamine. SPNs receive dopaminergic input from the substantia nigra and SPNs containing dopamine D1 receptors project back to the substantia nigra through the direct pathway (Gerfen et al., 1990), which is involved in initiating voluntary motor behavior as well as attention control (Tecuapetla et al., 2016; Agnoli and Carli, 2011). Learning deficits have been found to result when dopamine is blocked (Robinson et al., 2007; Robbins et al., 1990; Willuhn and Steiner, 2008). Previous

studies have also considered whether D1 receptors are required for the induction of LTP. Hawes et al. (2013) found that D1 receptors are required for LTP in the DM striatum using TBS. In another study, D1 receptors were found to be required for LTP in the DL striatum when using high-frequency stimulation (Kung et al., 2007). Other studies have further demonstrated the requirement of D1 receptors in striatal LTP, but the subregion of the striatum was not reported (Centonze et al., 2003; Calabresi et al., 2000). However, Park et al. (2014) found that blocking D1 receptors in the DL striatum does not prevent LTP using TBS. This apparent regional difference may be due to different stimulation protocols, which could recruit different molecular mechanisms, but it also may be due to a difference in dependence on D1 receptors for LTP in the two areas. No study has tested a regional difference in D1 receptor dependence, so we compared the effect of D1 receptor inhibition on LTP in the DM and DL striatum.

Traditionally, the studies that examine LTP in the rodent striatum have been performed with males only, but evidence suggests that females may have different rates of learning than males (Shors, 2016), and that learning may even vary among stages of the estrous cycle (Shors et al., 1998). Estradiol is a hormone that fluctuates during the estrous cycle, and even within the estrus stage itself (McLean et al., 2012) and has been found to impact LTP. Higher synaptic density has been found in the hippocampus during stages in which estradiol is elevated (Woolley and McEwen, 1992) and estradiol has been found to enhance LTP in the hippocampus (Grassi et al., 2011; Hasegawa et al., 2015). While the effect of estradiol on LTP in the striatum as a whole is not yet well understood, evidence indicates that it is necessary for LTP in the DL striatum using high-frequency stimulation

(Tozzi et al., 2015). It has also been shown to impair tasks that require striatal-based learning (Korol and Pisani, 2015). Progesterone, another hormone that fluctuates during the estrous cycle (McLean et al., 2012), may also impact LTP in the striatum. Higher levels have been found to impair LTP in hippocampal CA1 neurons (Foy et al., 2008), though research of progesterone's effect on corticostriatal LTP is limited. Because these two hormones fluctuate throughout the estrous cycle, the ability for corticostriatal LTP to occur may also fluctuate. Investigation into the ability for the DM striatum to undergo LTP in female mice under the same TBS treatment as male mice can give insight into whether female mice are appropriate subjects to use in future studies.

METHODS

Brain slices were obtained from male and female C57BL/6 mice between 50 and 150 days of age. The mice were anesthetized using isofluorane and their brains were extracted and placed into ice-cold slicing solution (KCl 2.8 mM, dextrose 10 mM, $NaHCO_3$ 26.2 mM, NaH_2PO_4 1.25 mM, CaCl 0.5 mM, Mg_2SO_4 7 mM, and sucrose 210 mM).

Estrus stage determination was done using a vaginal lavage immediately following brain extraction. A sample of vaginal fluid was extracted using a pipette and viewed at 450X with an American Optical 150 microscope. The criteria for estrus were a presence of numerous cornified epithelial cells and little to no nucleated epithelial cells or leukocytes.

A Leica vibratome was used to make 350 μm-thick slices of the striatum. Each slice was cut in half to separate the two hemispheres. The slices were incubated in oxygenated artificial cerebrospinal fluid (ACSF) (in mM: NaCl 126 mM, NaH_2PO_4 1.25 mM, KCl 2.8 mM, CaCl 2 mM, Mg_2SO_4 1 mM, $NaHCO_3$ 26.2 mM, and dextrose 11 mM) for 40 min at 33°C and then 40 min at room temperature (24-25°C).

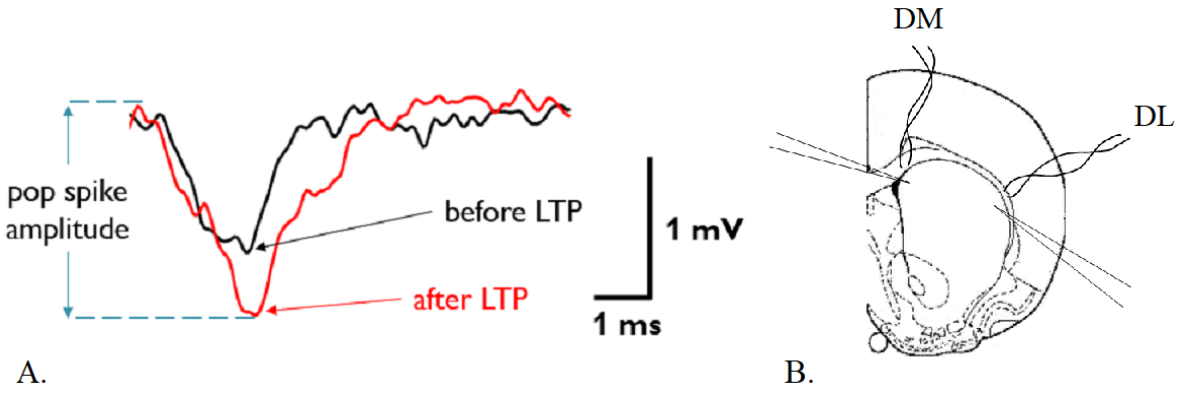

Fig. 1. Population spike / coronal slice of mouse brain: Fig. 1A. Recording electrodes were inserted into the striatum in the DM or DL striatum and bipolar stimulation electrodes were placed into the corpus callosum overlying the respective areas. The increase in pop spike amplitude from the black trace to the red trace indicates LTP has occurred. Fig 1B. Diagram of coronal slice of mouse brain (modified from Lipska et al., 2003).

During recordings, the slices were constantly perfused with 30-32°C oxygenated ACSF containing 50 μM picrotoxin to block gamma-aminobutyric acid A ($GABA_A$) receptors. The perfusion rate was 1.0 to 2.5 mL/min.

Current was injected using a twisted tungsten bipolar stimulating electrode into the white matter of the corpus callosum overlying the dorsomedial (DM) or dorsolateral (DL) striatum. Field recordings of the voltage responses from a population of neurons (pop spikes) in the DM or DL striatum were obtained using a borosilicate glass pipette containing ACSF (Fig. 1). The pop spikes were amplified using a Warner IE-251A amplifier. The recording software LabView was used to sample at 40 kHz.

Recording began with an input/output curve where 0.05 ms duration test pulses were given to the corpus callosum every 15 s. Test pulse currents ranged from 0.01 mA to

1.5 mA. The current that evoked a pop spike that was 40-60% of the maximum pop spike size was selected and used for the remainder of the recording.

The baseline recording was run with 30 s between each pulse for 15 min. In recordings using the addition of the drug SCH23390 to ACSF, the ACSF with the drug began flowing during the baseline for a full 15 min with the slice immersed in drug-containing ACSF.

After the baseline recording, the induction protocol was run using theta burst stimulation (TBS) with an inter-burst frequency of either 10.5 or 5.0 Hz (Fig. 2). During TBS, 10 trains of 10 bursts were delivered to the corpus callosum with either 0.095 s (10.5 Hz) or 0.2 s (5 Hz) between bursts. Each burst consisted of four pulses at 50 Hz. Each train was spaced 15 s apart.

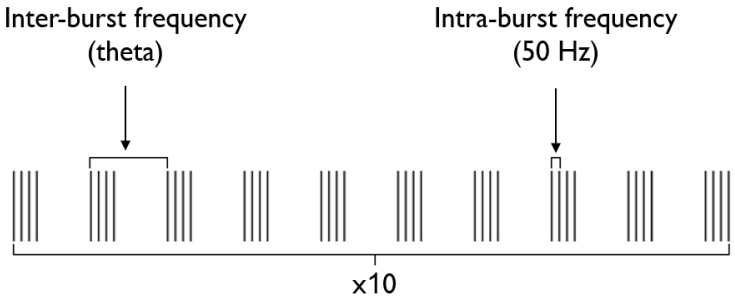

Fig. 2. Train of theta-burst stimulation: Bursts of 4 stimuli were given at a frequency of either 5 Hz or 10.5 Hz. Each train contained 10 bursts of stimuli. The entire TBS induction contained 10 trains of bursts, resulting in 400 total stimuli per induction.

Some slices were used for control recordings where instead of TBS, no stimulation was given for three minutes. For the drug treatment groups, the slices were continuously perfused with the drug-containing ACSF throughout the induction.

An hour-long follow-up was recorded following TBS, continuing 0.05 ms pulses every 30 s as during the baseline recording.

During post-processing, the baseline normalized pop spike size was calculated by averaging pop spike size every minute, dividing pop spike size by average baseline amplitude, and multiplying by 100 to achieve units of percent. Then, baseline slope was calculated by fitting a line to normalized amplitudes of the baseline. If the normalized baseline slope was found to exceed ±0.01 /min, the recording was excluded from the data. For the hour-long follow-up recording, the mean pop spike amplitudes at 25 to 30 min and 55 to 60 min were divided by the average baseline pop spike amplitude. The statistical analysis software JMP was used to run analyses of variance to compare LTP magnitude across groups.

RESULTS

We used field recordings in the dorsal striatum to determine which frequency of theta-burst stimulation (TBS) resulted in LTP of corticostriatal synapses in the dorsomedial (DM) and dorsolateral (DL) striatum. The amplitude of pop spike relative to baseline after 10.5 Hz and 5.0 Hz were compared.

To determine whether we can reproduce previous results (Hawes et al., 2013), we used 10.5 Hz TBS in the DM striatum. We were successfully able to produce potentiation at 30 min in the DM striatum using 10.5 Hz (N=11), while using 5.0 Hz TBS (N=8) did not increase pop spike size, confirming previous results (Fig. 3A). We observed a short-term depression with both frequencies that lasted for about 10 min; after this time, the pop spike amplitude after 5 Hz TBS was similar to that of the No TBS controls (N=8), indicating that no LTP occurred after 5 Hz TBS. To demonstrate this, we ran a generalized linear model (GLM) using the independent variables of TBS frequency and age as covariates [Fig. 3A; GLM, $F(2, 28) = 5.24$, $P = 0.012$]. Higher TBS frequency [GLM, $F(2,28) = 9.39$, $P = 0.005$] and lower age [GLM, $F(2,28) = 5.30$, $P = 0.029$) were associated with larger potentiation. However, the potentiation after 10.5 Hz TBS did not last until 60 min [Fig. 3.A; GLM, $F(2, 28) = 0.30$, $P = 0.74$], as it began to decay around 45 min. An effect of age was found where older mice exhibited lower LTP than younger mice using 10.5 Hz TBS at 30 min post induction. [Fig. 4A; GLM, $F(2,28) = 5.24$, $P=0.012$]. We found

that average age of male mice differed between our 10.5 Hz and 5 Hz groups [GLM, $F(2,28) = 11.28$, P=0.0001] and that our male DM 10.5 Hz group had the highest average age (Fig. 4B). This indicates that our groups were not homogeneous, and that we may have seen greater LTP in 10.5 Hz in the DM striatum had we used more mice under 100 days old.

To test our hypothesis that the DL striatum requires a lower TBS frequency than the DM striatum, we compared 10.5 Hz TBS with 5.0 Hz. In the 5.0 Hz group (N=5), we found an early depression that lasted about 20 min, but potentiation emerged by 30 min. Running a GLM using the independent variable TBS frequency as a categorical variable (age did not result in different LTP magnitudes dorsolaterally), LTP was significant at both 30 min [Fig. 3B; GLM, $F(2, 22) = 3.92$, P = 0.035] and 60 min [Fig. 3B; GLM, $F(2,22) = 4.35$, P = 0.025]. While 5.0 Hz resulted in LTP, 10.5 Hz (N=5) and the No TBS control (N=4) did not.

Although we did not achieve long-lasting LTP in the DM striatum, these data indicate that LTP in the DL striatum requires a lower TBS frequency than the DM striatum.

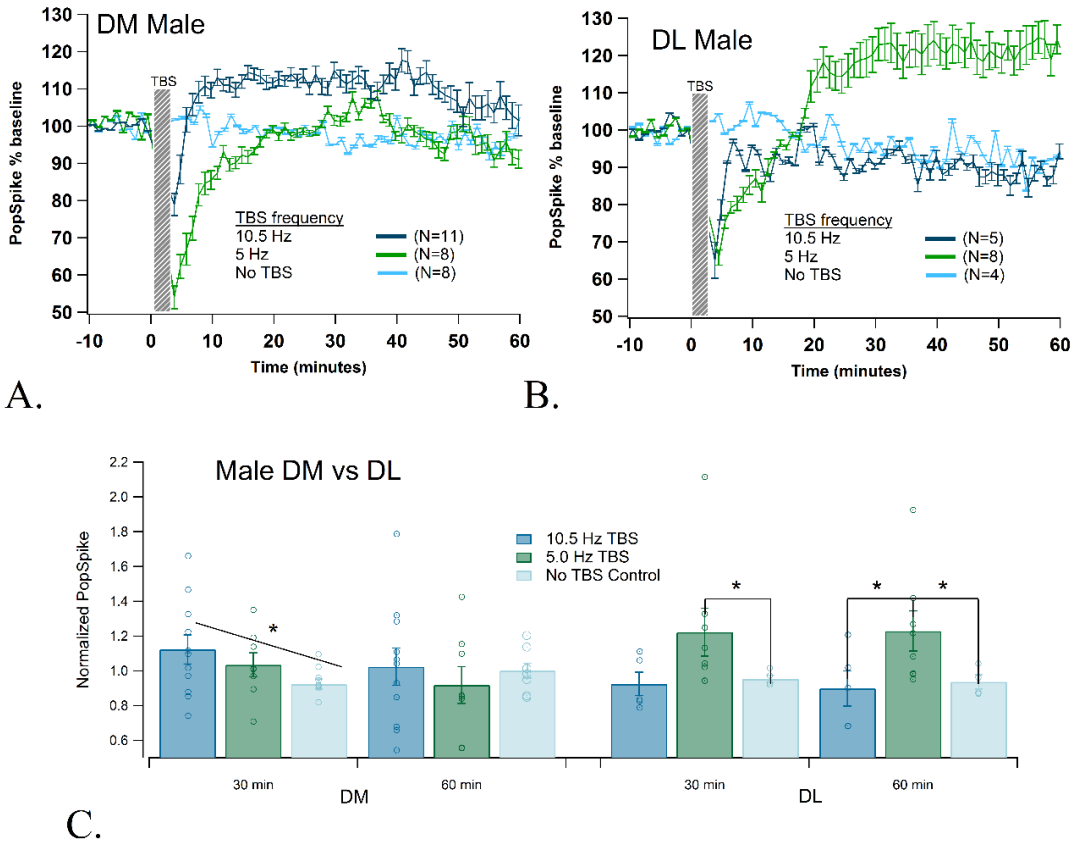

Fig. 3. Optimal TBS frequency for LTP induction differs between DM and DL striatum: Fig. 3A. 10.5 Hz TBS in the dorsomedial (DM) striatum induces LTP, while 5 TBS does not. Error bars represent ± standard error of the mean. Fig. 3B. 5 Hz TBS in the dorsolateral (DL) striatum induces LTP while 10.5 Hz TBS does not. Fig. 3C. Significant LTP was found at 30 min post induction using 10.5 Hz in the DM striatum and at both 30 and 60 min using 5.0 Hz in the DL striatum.

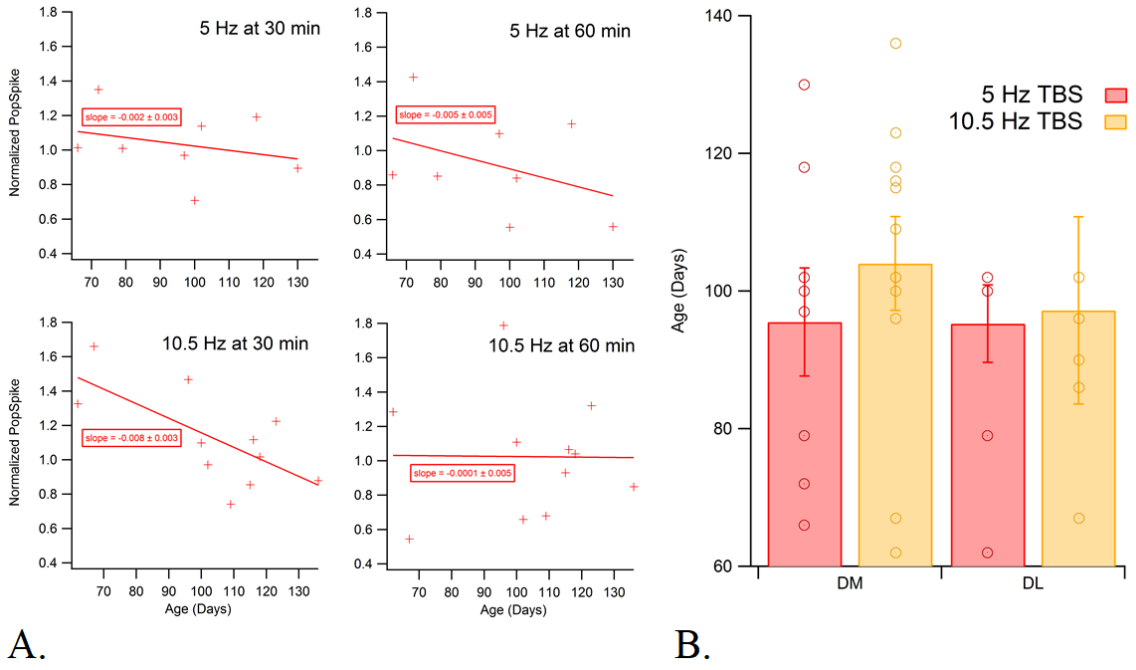

Fig. 4. Older mice exhibit reduced LTP in DM striatum: Fig. 4A. A negative relationship was observed between mouse age and LTP in the DM striatum at 30 min post induction. Fig. 4B. The DM 10.5 Hz TBS group had the highest average age of the DM groups.

We next examined whether D1 are necessary for LTP in the DL striatum in order to determine whether this area differs from the DM striatum in this regard. While Hawes et al. (2013) found that D1 receptors are required in the DM striatum, Park et al. (2014) found that they are not required in the DL striatum. This apparent regional difference could be due to differing stimulation protocols. To determine whether this is the case, we used Hawes' TBS protocol with a frequency of 5.0 Hz and perfused slices with ACSF containing the D1 receptor blocker SCH23390. We found that potentiation was not seen under this treatment (N=8), and furthermore, a small depression emerged. The No TBS control group

perfused with SCH23390 (N=4) exhibited similar pop spike size to the No TBS control group without the drug (N=4), indicating that this slight depression was not due to solely the drug. A GLM with drug as the independent variable demonstrated that blocking D1 receptors prevents LTP at both 30 min [Fig. 5; DLM, $F(3, 20) = 8.63$, P = 0.0076] and 60 min [Fig. 5; GLM, $F(3, 20) = 6.18$, P = 0.021]. This led us to conclude that D1 receptors are required for LTP in the DL striatum, similar to the DM striatum (Hawes et al., 2013).

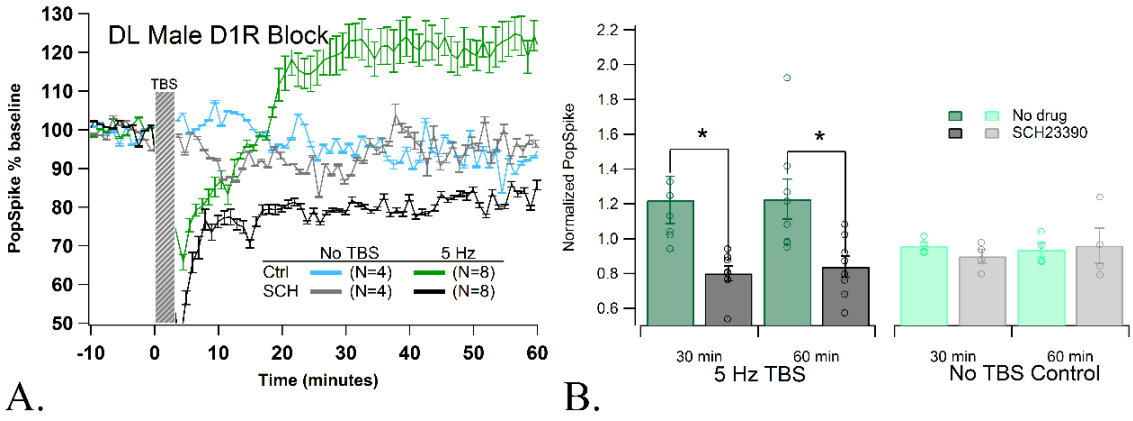

Fig. 5. Blocking D1 receptors prevents LTP in DL striatum: Fig. 4A. LTP was blocked in the DL striatum in the presence of the D1 receptor blocker SCH23390. Error bars represent ± standard error of the mean. Fig. 4B. A significant difference in potentiation was found between slices with no drug and slices with SCH23390 at both 30 min and 60 min post induction.

Due to limited past literature of electrophysiological experiments on female mice, it is unknown whether female mice demonstrate corticostriatal LTP in a similar fashion to male mice. Because females and males have been differences in learning (Shors, 2016), and because learning differences can also be seen among stages of the female estrous cycle

(Shors et al., 1998), we explored the question of whether effects of sex and estrus stage can also be seen in corticostriatal synaptic strengthening. We investigated whether females exhibit LTP similar to males under the same TBS protocol by using 10.5 Hz TBS on the DM striatum of female mice who were not in the estrus stage and comparing the LTP magnitude to that of males. We found that, after 10.5 Hz TBS, non-estrus females (N=12) exhibited similar LTP to males. Unlike in males, the LTP seen in non-estrus females lasted until 60 min. The No TBS control in non-estrus females (N=13) did not potentiate, though a slight potentiation was seen around 45 min, indicating that the baseline was not stable as we had had thought. 10.5 Hz TBS was also used in estrus females (N=10) and we found that this did not result in LTP. The estrus No TBS control (N=7) also did not result in LTP and had a slight depression. A GLM with sex, TBS frequency, and age as independent variables found that LTP at 30 min significantly differed among sex, TBS frequency, and age, where lower ages exhibited larger LTP [Fig. 6; GLM, $F(4, 56)=5.23$, $P=0.001$]. At 60 min, a GLM was used with sex as the independent variable, and sex was once again significant [GLM, $F(2, 58) = 3.54$, $P = 0.035$]. Therefore, we concluded that non-estrus females exhibit similar LTP to males under the same induction protocol and estrus females do not.

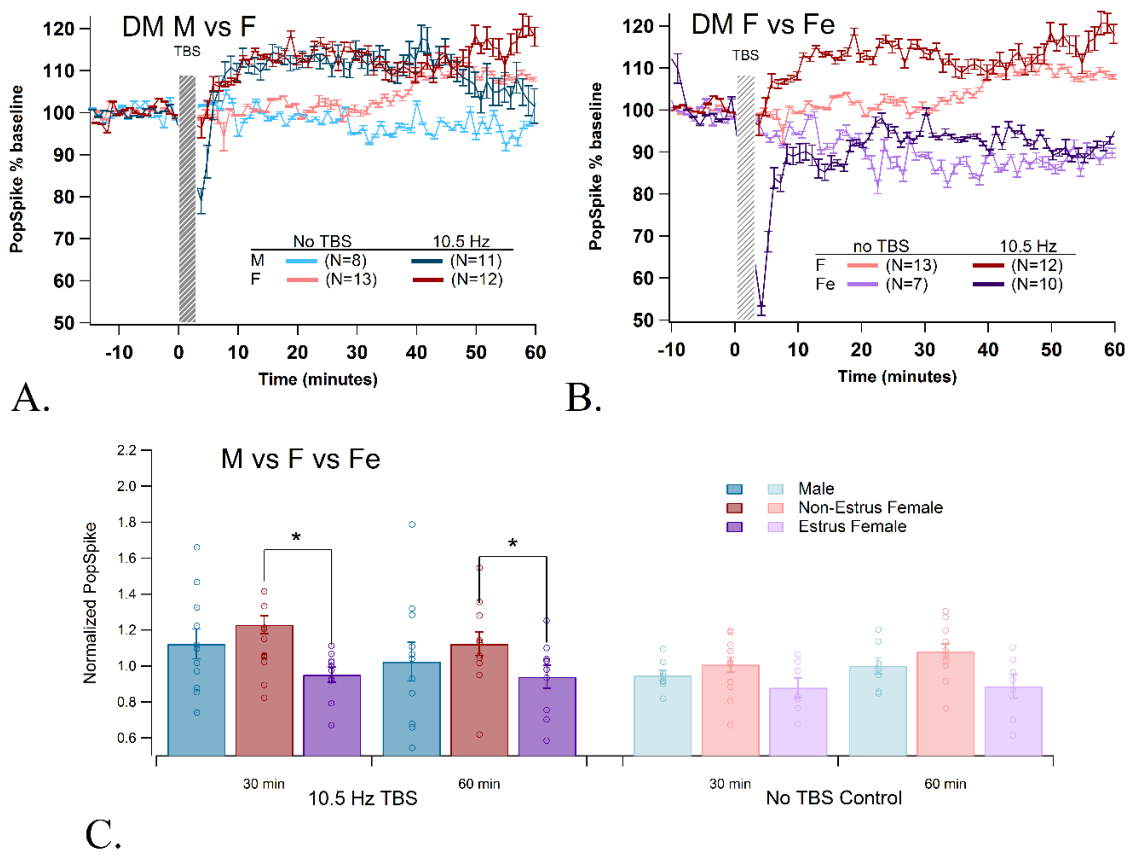

Fig. 6: LTP induction in non-estrus females, but not estrus females, is similar to males: Fig. 5A. Males (M) and non-estrus females (F) exhibit similar LTP after 10.5 Hz TBS in the DM striatum. Error bars represent ± standard error of the mean. Fig. 5B. Estrus females (Fe) do not exhibit LTP after 10.5 Hz TBS in the DM striatum. Fig. 5C. A significant difference in potentiation was found between non-estrus females and estrus females at both 30 min and 60 min post induction.

DISCUSSION

We demonstrated that the DM and DL striatum differ in sensitivity to different TBS burst frequencies but not to the requirement for D1 receptors. We found that a lower TBS frequency is required for LTP in the DL striatum than the DM striatum. The frequencies that produce LTP in the DM and DL striatum were 10.5 Hz and 5.0 Hz, respectively. These correspond to the frequencies to which striatal neurons were found to be naturally entrained (Thorn and Graybiel, 2014). We found that LTP in the DL striatum is dependent on D1 receptors. Because this dependence has also been found in the DM striatum (Hawes et al., 2013), this rules out the possibility that this receptor is responsible for differences in LTP between the DM and DL striatum.

In general, the dependence of LTP on dopamine receptors has been well established in the striatum. The activation of D1 receptors initiates an intracellular signaling pathway involving adenylyl cyclase, which results in the production of cycle adenosine monophosphate (cAMP), which activates protein kinase A (PKA) (Cerovic et al., 2013). PKA is necessary for LTP in the striatum, as inactivating PKA prevents LTP (Spencer and Murphy, 2002; Hawes et al., 2013). PKA produces LTP through several mechanisms, such as the phosphorylation of postsynaptic α-amino-3-hydroxy-5-methyl-isoxazoleproprionic (AMPA) receptors (Hell, 2016; Nayak et al., 1998). Our findings support the notion that the DL striatum also has this requirement of D1 receptors in order to undergo LTP. Given

these observations, the reason Park et al. (2013) did not find dependence of DL LTP on dopamine could lie in the difference in their stimulation protocol. Their usage of an intra-burst frequency of 100 Hz could have recruited additional molecular mechanisms that facilitate in producing LTP. It has been demonstrated in hippocampal models that higher stimulation frequencies can activate certain molecular pathways due to a higher influx of calcium that lower frequencies cannot achieve (Jedrzejewska-Szmek et al., 2017). Perhaps Park activated more calcium-calmodulin-dependent kinase II (CaMKII) than we did through their 100 Hz bursts. CaMKII has been implicated in LTP through upregulation of AMPA receptors in synapses in the hippocampus (e.g. Hayashi et al., 2000), though its role in corticostriatal LTP is not yet well understood. Park also used a shorter inter-train interval, which has been shown to result in lower dopamine release (Mamaligas et al., 2016), which could contribute to greater dependence on CaMKII rather than PKA (Kim et al., 2010). Therefore, Park's stimulation protocol could have circumvented dopaminergic signaling pathways in LTP by activating additional CaMKII.

Our observation that different TBS frequencies are required for LTP in the DM and DL striatum has implications for understanding the mechanisms that produce these differences. Several heterogeneities have already been noted in the dorsal striatum. For example, previous research into differences between the DM and DL striatum has shown that there is a higher concentration of endocannabinoids in the DL striatum, which may help explain why it LTD is easier to find in this area (Herkenham et al., 1991; Gerdeman et al., 2002; Hawes et al., 2013). Dopamine reuptake has also been found to differ between the two areas due to a higher expression of dopamine transporter (DAT) in the DL striatum

(Wickens et al., 2007), so this area requires more dopamine release than the DM striatum in order to achieve the same amount of dopamine in the synapse. Bioinformatics approaches, such as the Allen Brain Atlas (http://mouse.brain-map.org/gene/show/92636), may help to identify other molecules with medial-lateral gradients. Manipulation of signaling pathways that differ between the DM and DL striatum can provide a means to influence types of learning that are specific to these striatal regions in order to enhance or inhibit the behaviors with which they are associated.

Another difference that we observed between the two regions involved the time course it takes to achieve LTP. Potentiation took longer to develop in the DL striatum than the DM striatum; while LTP emerged within 10 minutes after TBS in DM slices, it was delayed almost twice as long in DL slices, emerging an average of 20 minutes after TBS. This could relate to the time course of a larger scale where habitual behaviors take longer to develop than goal-directed behaviors (Corbit et al., 2012; Costa, 2007). Consistent with this difference, DM spiny projection neuron (SPN) activity is observed in earlier stages of skill-learning while DL SPN activity is observed in later stages (Yin et al., 2009).

We found a trend in which younger mice tended to exhibit LTP of a greater magnitude than older mice. Age effects on LTP have been shown in the hippocampus of postnatal mice (Dumas, 2012) and middle-aged rats (Rex et al., 2005). It has also been shown in the striatum of postnatal rats (Partridge at al., 2000). Many different molecules change as rodents age, and some of these changes could lead to decreased ability for corticostriatal synapses to potentiate. D2 receptor turnover rates decrease with age (Leff et al., 1984) as well as D2 receptor expression (Gerald et al., 1998). D2 receptors have been

associated with LTD (Cerovic et al., 2013). Ras homolog gene family, member A (RhoA) activity, demonstrated to be necessary for hippocampal LTP, decreases with age (Kang et al., 2013; Briz et al., 2015; Rex et al., 2009). Older mice exhibit increased expression of opioid receptors with age (Gerald et al., 1998). Upregulation of opioid receptor expression could impair LTP as evidenced by κ-opioid receptor activation's effect of reducing dopamine release (Hawes et al., 2017). CaMKII binding to the PP1-binding protein spinophilin also increases with age (Baucum II et al., 2012). PP1 deactivates CaMKII, so this enhanced binding may have a detrimental effect on LTP (Shioda and Fukunaga, 2018; Blitzer et al., 1998). A combination of these changes may explain why this trend of decreased LTP with age was seen.

Our study compared LTP in the DM striatum of female mice who are not in estrus with that of female mice in estrus and found that those in estrus do not exhibit LTP. This supports previous findings that striatal-based learning is impaired in rats during the estrus stage (Korol and Pisani, 2015). The dynamic levels of estradiol and progesterone (McLean et al., 2012) raise the question of whether these hormones are influencing pathways that can impact LTP. Estradiol has been found to increase the release of dopamine in the dorsal striatum (Shams et al., 2016) and to inhibit dopamine uptake through the dopamine transporter (Watson et al., 2006). In the hippocampus, estradiol facilitates activation of CaMKII (Sawai et al., 2002). These effects should enhance LTP, however estradiol also facilitates dopamine binding to D2 receptors (Bazzett and Becker, 1994), which is linked to LTD (Cerovic et al., 2013), thus this indicates that it should reduce LTP. Progesterone also increases dopamine release in the striatum (Petitclerc, 1995). Its metabolite

allopregnanolone is known to enhance the activity of $GABA_A$ receptors (Belelli et al., 2002), though we blocked these receptors with picrotoxin. A limitation to understanding how these hormones may be affecting LTP is the fact that a consensus does not exist on their levels during estrus. Some studies claim estrogen is raised at estrus (Wood et al., 2007; Rychlik et al., 2001), while others claim this is the stage with lowest levels of estrogen (Korol & Pisani, 2015; Warren et al., 1995). Some claim progesterone is elevated during estrus (Murphy & Segal, 2000; Corpéchot et al., 1997), while others claim it is lowest at estrus (Wood et al., 2007; Warren et al., 1995). Still others argue that estrus is a period where the concentrations are in the process of changing (McLean et al., 2012). If raised estradiol inhibits LTP, then a similar effect would also be expected during the stage of proestrus, where high estradiol is observed (Shors et al., 1998). Likewise, raised progesterone is also seen during metestrus (Corpéchot et al., 1997). Additionally, LTP may be impacted differently in the DL striatum. We only tested dorsomedially, but E2 receptor activation by estradiol has been shown to be necessary for LTP in this area (Tozzi et al., 2015), so perhaps estrus would aid LTP in this area. In future research, investigation into the molecular mechanisms of the influence of estrogen on LTP can give insight into the role of the estrous cycle on learning.

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BIOGRAPHY

Valerie J. Lewitus graduated from W. T. Woodson High School in Fairfax, Virginia in 2010. She received her Bachelor of Science in Psychology from the University of Mary Washington in 2014 and served as the Undergraduate Academic Advisor in the Interdisciplinary Program in Neuroscience at George Mason University from 2017 to 2018.