USING PATCH CLAMP ELECTROPHYSIOLOGY TO DETECT CHANGES IN EXCITATORY SYNAPTIC STRENGTH IN THE STRIATUM OF RATS TREATED WITH METHAMPHETAMINE

by

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B.S. Chemistry, Sewanee: The University of the South, 2013

A Thesis Submitted to the Faculty of the Mercer University School of Medicine in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE IN BIOMEDICAL SCIENCES

Macon, GA

2017
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ACKNOWLEDGMENTS

I would like to thank Dr. Andon Placzek of Mercer University for his guidance and support during this study, Jordan Logue for his help with learning the techniques and helping with the study, Dr. Ashley Horner for her insight into stereotypy and METH use, and Dr. Rick McCann for organizing the Biomedical Sciences Program.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ACKNOWLEDGMENTS</th>
<th>iii</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2. METHODOLOGY</td>
<td>12</td>
</tr>
<tr>
<td>Drugs</td>
<td>12</td>
</tr>
<tr>
<td>Animals</td>
<td>12</td>
</tr>
<tr>
<td>Slice Electrophysiology</td>
<td>13</td>
</tr>
<tr>
<td>Immunohistochemistry and Visualization</td>
<td>15</td>
</tr>
<tr>
<td>3. RESULTS</td>
<td>17</td>
</tr>
<tr>
<td>AMPAR/NMDAR Ratios in Naïve and Saline Treated Rats</td>
<td>17</td>
</tr>
<tr>
<td>AMPAR/NMDAR Ratios in Chronic METH Treated Rats</td>
<td>18</td>
</tr>
<tr>
<td>4. DISCUSSION</td>
<td>23</td>
</tr>
<tr>
<td>Basal AMPAR/NMDAR Ratios Do Not Differ Between Naïve and Saline Controls for Either Striatal Subregion</td>
<td>23</td>
</tr>
<tr>
<td>Chronic METH Treated Rats Show An Increase In Patch Neuron AMPAR/NMDAR Ratios, Suggesting LTP</td>
<td>24</td>
</tr>
<tr>
<td>5. FUTURE DIRECTIONS</td>
<td>25</td>
</tr>
<tr>
<td>6. CONCLUSIONS</td>
<td>28</td>
</tr>
<tr>
<td>7. REFERENCES</td>
<td>29</td>
</tr>
</tbody>
</table>
Methamphetamine (METH) abuse is quickly becoming an epidemic in both the United States and the world. One symptom of METH use is stereotypic behavior, or repetitive, non-goal oriented behaviors that interfere with goal directed behaviors. Investigators have looked at the striatum for the formation of these behaviors since ritualistic behaviors are defining characteristics of this region. The ventral striatum is associated with limbic circuits while the dorsal striatum is linked to motor circuits. Within these regions lie two additional subregions, the patch and the matrix. The patch receives predominantly limbic inputs while the matrix has predominately sensorimotor inputs. Previous work has determined that the patch
region of the dorsal striatum is responsible for the formation of stereotypic behavior, but the precise mechanism remains unclear. We hypothesized that these behaviors arise from synaptic plasticity occurring in the patch and matrix regions of the dorsal striatum. Chronic METH was given to rats and electrophysiology was used to determine changes in excitatory synaptic strength in neurons within this brain region. Although we did not find any statistically significant difference when comparing the patch and matrix neurons of saline and chronic METH treated animals respectively, we did see a trend towards long term potentiation in chronic METH patch treated neurons. More studies will need to be done to increase the sample size and determine if the synaptic changes are long-term or short-term in nature.
INTRODUCTION

Methamphetamine (METH) use in the United States is a rising problem linked to the ease by which METH can be produced as well as the addictive nature of the drug. The first widespread use of METH was during World War II, where the benefits of the drug (increased alertness and aggression, decreased hunger and need to sleep) were shown to increase combat effectiveness (Rusyniak 2011). At the time, the toxic effects of METH were not well understood, and many soldiers from Germany, Japan, and the United States were given this drug. When they returned home after the war, many were addicted to METH. Japan was the first of these countries to experience a true epidemic of METH abuse (Rusyniak, 2011).

METH is easier to produce than other drugs of abuse, such as cocaine and heroin. While cocaine must be made from plant extracts, METH can be synthesized from supplies found in any pharmacy nationwide and made in a bathtub or even a tail pipe (this is how it received its nickname “crank”) (Rusyniak, 2011). In 2005, in an effort to limit the production of METH in the United States, Congress passed the Combat Methamphetamine Epidemic Act. This act was directed at any drugs that contained ephedrine, pseudoephedrine, or phenylpropanolamine, which are sources of the reagents needed for the manufacturing of METH (CMEA, 2005). In order to control access to these drugs, an ID became required in order to purchase the drugs
and only 7.5 grams could be bought every 30 days. This legislation also moved these drugs “behind the counter,” which kept the drugs in a safer place until purchase occurred (CMEA, 2005). After the law was put in to effect, the number of admissions to hospitals for METH abuse declined for the first time in 10 years (Rusyniak, 2011). However, since 2011, the number of new initiates (people who have used METH for the first time that previous year) in the United States has been steadily increasing again (Substance Abuse, 2014). In 2008, the United Nations Office on Drugs and Crime estimated that 25 million people worldwide abused METH, which was greater than the numbers for both cocaine (14 million) and heroin (11 million) (Rusyniak, 2011).

METH has different physiological and behavioral effects in humans depending on whether it is used acutely or chronically. When taken acutely, several major effects are related to central nervous system (CNS) stimulation: euphoria, increased alertness, increased aggression, decreased hunger, and decreased need to sleep. In a clinical setting, acute effects of METH can be seen in terms of increased sympathetic nervous system responses: vasoconstriction, bronchodilation, and increased heart rate and blood pressure (Rusyniak, 2011). Overdoses of METH can cause hyperthermia, which can sometimes be extreme, leading to death. When used chronically, there are pronounced effects that persist even when not actively taking the drug. These include anxiety, depression, psychosis, and decreases in episodic memory, motor function, and executive function. Psychosis is the most common reason for doctors to see chronic METH users because the signs and symptoms of
psychosis tend to mimic those seen in schizophrenia, including paranoia and auditory hallucinations (Rusyniak, 2011). A common manifestation of this psychosis is called formication, or “METH mites.” This refers to a sensation similar to insects crawling on a user’s skin, often leading them to scratch at themselves. Along with poor hygiene, this can result in scars and open wounds on a user. Cognitive dysfunction, such as diminished episodic memory and executive function, contribute to METH addiction by limiting the ability to recall past mistakes and decreasing control over the urge to use METH. Since executive function is hindered, METH users have trouble when they try to break the habit. Instead, they continue to use METH, even in situations where they know that they should not and where it might be harmful or dangerous. While pathological habit formation (such as that which occurs in METH addiction) is not well understood, research into the mechanisms underlying these pathological habits is key in the prevention of METH addiction.

The Diagnostic and Statistical Manual of Mental Disorders (DSM-V) lists multiple criteria for Stimulant Use Disorder (American Psychiatric Association, 2013). Of the 11 criteria, 4 are related to the formation of a habit:

1) There is a persistent desire or unsuccessful efforts to cut down or control stimulant use.

2) Recurrent stimulant use resulting in a failure to fulfill major role obligations at work, school, or home.
3) Continued stimulant use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the stimulant.

4) Recurrent stimulant use in situations in which it is physically hazardous (American Psychiatric Association, 2013).

Stimulants are capable of habit formation, causing the user to discount adaptive behavior, such as pursuing healthy social interactions or avoiding dangerous situations, in order to fulfill the internal need for the drug. With increasing and repetitive METH use, there is loss of control and neuroplasticity in multiple brain structures, including those implicated in habit learning. The dorsal striatum plays a key role in the formation of habits and is believed to play a role somewhere in-between the first use of a drug and the eventual habit (Koob and Volkow, 2010). Other areas of the brain involved in the formation of addiction, such as the ventral tegmental area (VTA) and the ventral striatum, have been well studied, and a key feature that occurs in these areas is drug induced synaptic plasticity (Hyman et al, 2006, Kauer and Malenka, 2007). Therefore, it is reasonable to look into synaptic plasticity in the dorsal striatum as a potential mechanism of habit learning in drug addiction.

METH works in the brain by entering dopamine neurons via dopamine transporters on the presynaptic terminal. METH then causes vesicular monoamine transporters to dump dopamine into the synaptic cleft, causing an increase in the amount of postsynaptic dopamine signaling (Hyman et al, 2006). The cortical
afferents that project to the dorsal striatum are excitatory (glutamatergic), while the primary neurotransmitter released by the medium spiny neurons (MSNs) of the dorsal striatum is gamma-aminobutyric acid (GABA). Dopaminergic neurons modulate the release of glutamate onto the GABAergic neurons, changing the firing frequencies of these neurons (Hyman et al, 2006), modifying their inhibitory output. The primary behavioral function of dorsal striatum MSNs is in the selection and initiation of motor and emotional “programs” in response to afferent cortical and thalamic activity (Crittenden and Graybiel, 2011).

One characteristic of METH abuse is the occurrence of stereotypy. Stereotypy is defined as inflexible, repetitive, purposeless behavior that interferes with the ability to initiate normal adaptive responses (Horner et al, 2010, Murray et al, 2014). Stereotypic movement is observed in both humans and experimental animals when given a relatively high dose of METH. In humans, a type of stereotypic behavior called “punding” also occurs in about 40% of METH users (Rusyniak, 2011). One example of punding is that of a clock maker who takes a clock apart and then puts it back together over and over again. It is a repetitive process that a person usually performs multiple times in a row (Rusyniak, 2011). The repetitive nature of both stereotypy and punding has caused many investigators to look to the dorsal striatum for a potential cause for these behaviors (Canales and Graybiel, 2000, Horner et al, 2010, Horner et al, 2012). Furthermore, previous work has shown that the dorsal striatum is key in the formation of addiction, where habitual, ritualistic behavior and motivational states are defining features (Yager et al, 2015).
Broadly speaking, the striatum can be functionally and anatomically divided into two regions, the ventral striatum and the dorsal striatum. The ventral striatum is mainly associated with the limbic system while the dorsal striatum is linked more with motor circuits (Crittenden and Graybiel, 2011). Within the motor control pathways of the dorsal striatum there are two main anatomical circuits, the direct pathway and the indirect pathway. The direct pathway leads to disinhibition of the thalamus, allowing for the initiation of movement. The main neurotransmitter used in this pathway is GABA. The other pathway, the indirect pathway, leads to net inhibition of the thalamus (dis-inhibition), repressing the initiation of movement. Glutamate is the main neurotransmitter for the indirect pathway (Yager et al, 2015).

Separate from the direct and indirect pathways are subsections within the dorsal striatum known as the patch (or striosomes) and the matrix. These two compartments can be separated based on neuropeptide expression, cell-surface receptors, and connectivity (Murray et al, 2014). Patch compartment MSNs express mu opioid receptors while those in the matrix do not. The majority of the dorsal striatum consists of the matrix compartment which primarily receives input from sensorimotor regions. However, the patch compartment has predominantly limbic inputs, such as the prelimbic cortex and amygdala (Murray et al, 2014). Both of these subregions have outputs to the direct and indirect pathways (Figure 1). With these differences in mind, experiments to determine what role each compartment may have in the formation of addiction and the behavioral aspects of stereotypy have been performed (Horner et al, 2010, Horner et al, 2012, Murray et al, 2014).
Work in the Horner lab has helped elucidate the role of the patch and matrix in rats given METH. In these studies, the patch compartment was ablated using dermorphin and saporin (DERM-SAP). DERM binds to mu opioid receptors on the patch compartment neurons and causes internalization of the receptors. SAP is a ribosome inactivating cytotoxin that can only work when internalized by the
neuron. This leads to the death of the patch compartment while keeping the matrix intact. Some rats in these studies were infused with only SAP (as a control) while others were infused with DERM-SAP to selectively lesion patch neurons. The rats were then given METH and were watched for stereotypic behavior, spatial confinement, and locomotor activity. Rats who received DERM-SAP or SAP and were not given METH were used as an additional control group. When the patch compartment was ablated, there was a significant decrease in the level of stereotypic behavior as well as a decrease in spatial confinement (they explored their cage more) and an increase in locomotor activity (they moved around more). These studies suggest that there is a link between stereotypic behavior formation and an intact patch compartment (Murray et al, 2014).

Synaptic plasticity is a way in which neurons can change the strength of synaptic transmission between themselves. There are multiple forms of synaptic plasticity, and two commonly studied forms include long-term potentiation (LTP) and long-term depression (LTD). LTP is the strengthening of synaptic transmission that last for at least an hour, and has been shown to be an important mechanism underlying learning and memory. LTD is the long-lasting weakening of synaptic transmission that also plays an important role in learning and memory (Malenka and Bear, 2004). One way to measure changes in synaptic strength is to evoke excitatory postsynaptic currents (EPSCs) in neurons. There are two main subtypes of receptors for glutamate in the dorsal striatum: the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and the N-methyl-D-aspartic acid
receptor (NMDAR). In whole-cell voltage-clamp experiments, NMDARs cannot activate at a negative holding potential (such as -70 mV) due to voltage-dependent blockade by Mg\(^{2+}\) ions. In contrast, AMPARs can activate at these negative holding currents, allowing for a fast excitatory (depolarizing) response from the neuron. At a positive holding potential (e.g., +40 mV), it is possible to measure the response of both the AMPAR and NMDAR components of the evoked EPSC, resulting in a dual component EPSC. NMDARs can be blocked using DL-2-Amino-5-phosphonovaleric acid (DL-AP5), leaving only the AMPAR current. By subtracting the AMPAR current from the dual component EPSC, the ratio of AMPAR to NMDAR peak currents can be determined. If this ratio increases, it means that more AMPARs have been placed on the postsynaptic membrane, increasing the fast component of the EPSC. This represents the formation of LTP (Figure 2). If the ratio decreases, LTD is occurring, manifested by fewer AMPARs present on the postsynaptic membrane (Kauer and Malenka, 2007).
Figure 2. An illustration of LTP formation within a VTA dopamine neuron. By calculating the ratio of AMPAR to NMDAR, it is possible to determine the relative strength of synaptic currents. EPSC currents can be determined by subtracting the NMDAR current from the dual component EPSC, leaving the AMPAR current. A) An illustration of a synapse under basal or control state with the separate EPSC currents shown below. B) The formation of LTP occurs by an increase in the number of AMPAR placed on the post-synaptic membrane (Kauer and Malenka, 2007).

The purpose of this study was two-fold. First, we hypothesized that METH induces LTP in the patch compartment of the dorsal striatum, resulting in increased stereotypy due to enhanced excitatory signaling within striatal circuitry linking
movement with internal emotional states. Second, we hypothesized that METH induces LTD in the matrix compartment of the dorsal striatum, resulting in decreased locomotion due to decreased excitatory signaling in striatal circuits underlying purposeful, goal-directed movement.
METHODOLOGY

Drugs

(±) METH-HCl was provided by the National Institute on Drug Abuse. METH (or volume-matched saline as a control) was injected subcutaneously at a volume of 1mL/kg body weight. The dose of METH given was at 15 mg/kg body weight based on previous work by Dr. Horner (Horner et al, 2010; Murray et al, 2014).

Animals

Male Sprague Dawley rats from Charles River Laboratories (Wilmington, MA) weighing 350-550 g were used in all experiments. Rats were singly housed in plastic cages in a temperature – controlled room on a 14:10 hour light/dark cycle with free access to food and water.

Rats were divided into four groups one week after arrival at our facility. One group was used as the naïve control group. Another group was given a 1 mL/kg injection of saline 2 hours pre-sacrifice on the day of the study (saline rats). The third group was injected with METH 2 hours pre-sacrifice on the day of the study (acute METH rats). The final group was injected with METH once a day for seven days before the study day (chronic METH rats).
Slice Electrophysiology

Rats were anesthetized in a chamber with 1 mL of isoflurane included. The animals were then decapitated with the use of a guillotine. The brain was removed, trimmed, and glued with the ventral surface up to a block of 4% agar attached to the vibratome tray. Horizontal slices of 225 μm thickness were cut using a vibratome (Compresstome; Precisionary Instruments). Unless otherwise noted, all reagents and experimental compounds were obtained from Sigma-Aldrich (St. Louis, MO). The slices were cut in a room temperature solution containing the following (in mM): 92 NMDG, 2.5 KCl, 1.25 NaH2PO4, 30 NaHCO3, 20 HEPES, 25 glucose, 2 thiourea, 5 Na-ascorbate, 3 Na-pyruvate, 0.5 CaCl2·4H2O, and 10 MgSO4·7H2O. This solution was then titrated to a physiological pH using concentrated hydrochloric acid (Ting 2014). After cutting, the slices were placed in hot water bath (34 °C) containing the cutting solution. After 10 minutes, slices were moved to a beaker containing a solution at room temperature. This solution contains the following (in mM): 92 NaCl, 2.5 KCl, 1.25 NaH2PO4, 30 NaHCO3, 20 HEPES, 25 glucose, 2 thiourea, 5 Na-ascorbate, 3 Na-pyruvate, 2 CaCl2·4H2O, and 2 MgSO4·7H2O and saturated with 95% O2 / 5% CO2 (Ting 2014). Slices were allowed to recover for 30 minutes before recording. Slices were submerged in a chamber perfused continuously with oxygenated standard artificial cerebrospinal fluid (ACSF) (2-3 mL/min), containing picrotoxin (100 μM, Tocris Bioscience, R&D Systems, Minneapolis, MN) at about 32 °C. Picrotoxin was added to block GABAR-mediated IPSCs. Medium spiny neurons of the dorsolateral striatum were visualized under a Scientifica SliceScope (Uckfield,
Sussex, UK) with Olympus optics set for differential interference contrast optics and equipped with a 40x water-immersion lens. A bipolar tungsten stimulating electrode connected to a constant current stimulus isolator (A-365, World Precision Instruments, Sarasota, FL) was placed laterally 50-150 μm away from the cell of interest. To evoke excitatory postsynaptic currents (EPSCs), stimuli (100 microseconds) were delivered at 0.05 Hz. The stimulation strength was set to yield EPSCs with peak amplitudes of 100-200 pA.

Whole-cell patch-clamp recordings were performed with thin-walled borosilicate glass micropipettes (3-5 MΩ) (TW150F-4, World Precision Instruments, Sarasota, FL). Cells were voltage clamped at -70 mV. The liquid-junction potential was not compensated. The pipette solution contained (in mM): 117 CsMeSO\textsubscript{3}; 0.4 EGTA; 20 HEPES; 2.8 NaCl; 2.5 ATP-Mg 2.0; 0.25 GTP-Na; 5 TEA-Cl; adjusted to pH 7.3 with CsOH and 290 mOsmol/L. Data were obtained with a PC-505B amplifier (Warner Instruments, Hamden, CT), digitized at 10 kHz with a Digidata 1440A (Molecular Devices, Sunnyvale, CA), recorded using Clampex 10, and analyzed with Clampfit 10 software (Molecular Devices, Sunnyvale, CA). Recordings were filtered online at 5 kHz with a Bessel low pass filter. A 2 mV hyperpolarizing pulse was applied before each EPSC to determine the access resistance (Ra). Data were discarded if the Ra was unstable or greater than 25 MΩ, holding current was above 400 pA, input resistance dropped more than 20% during the recording, or EPSC amplitudes changed more than 20%. AMPAR/NMDAR ratios were determined as follows. Neurons were then voltage clamped at +40 mV until the holding current
stabilized (<400 pA). After recording the dual component EPSC, dL-AP5 (100 μM, Tocris Bioscience, R&D Systems, Minneapolis, MN) was bath applied for 10 minutes to block NMDAR. An average of 10-15, baseline adjusted sweeps were used as pre- and post-dL-AP5-treated currents. The AMPAR component was measured and the NMDAR component was determined by offline subtraction of the AMPAR component from the dual component EPSC using Clampfit analysis software. The peak amplitudes of the separate components were used to calculate the AMPAR/NMDAR ratios.

Immunohistochemistry and Visualization

After recording, slices were rinsed three times in 0.1 M phosphate buffered saline (PBS). Slices were then fixed in 10% neutral paraformaldehyde overnight. Slices were rinsed three more times in 0.1 M PBS for 24 hours then blocked with a solution containing 3% normal goat serum and 0.3% Triton X-100 (dissolved in 0.1 M PBS) for 2 hours at room temperature. Slices were incubated with primary antibody (rabbit anti-mu opioid receptor (MOR) at a 1:1000 dilution in 0.1 M PBS containing 0.3% Triton X-100 and 0.1% bovine serum albumin) overnight on a shaker at 4 °C. The next day, slices were rinsed with PBS 3 times for 15 minutes each at room temperature. Slices were then incubated with secondary antibody (Cy3 – conjugated donkey anti-rabbit IgG 1:200 diluted in 0.1 M PBS containing 0.3% Triton X-100 and 0.1% BSA) and antibody for neurobiotin labeling (AMCA-conjugated streptavidin DyLight 488 at 1:1000 to secondary antibody solution) and placed on a shaker overnight at 4 °C. Slices were rinsed 3 times with PBS for 15
minutes each and then mounted on slides using VectaShield anti-fade medium.

Slices were visualized using either a Zeiss Apotome Fluorescence Microscope (Thornwood, NY) or a Nikon C1 Laser-scanning Confocal Microscope (Melville, NY)
RESULTS

AMPAR/NMDAR Ratios in Naïve and Saline Treated Rats

When comparing the two distinct subregions (patch vs. matrix) of the dorsal striatum, we did not see a statistically significant difference in the AMPAR/NMDAR ratios from naïve rats compared to saline injected rats (matrix AMPAR/NMDAR ratios: naïve, 1.2423 ± 0.1589; saline, 1.0116 ± 0.1492) (Figure 3). This suggests that saline injections alone had no effect on excitatory synaptic transmission in dorsolateral striatal MSNs. However, we did see that the patch compartment MSNs generally have a trend toward higher ratio than the matrix neurons (patch AMPAR/NMDAR ratios: naïve, 1.3415 ± 0.6902; saline, 1.5901 ± 0.6507) (Figure 3), although this difference was not statistically significant.
Figure 3. Basal AMPAR/NMDAR ratios observed in untreated (naïve) and saline-injected patch and matrix MSNs within the dorsal striatum. No statistically significant differences were observed between groups (n = 5 cells per group).

AMPAR/NMDAR Ratios in Chronic METH Treated Rats

When compared to saline injected animals in the matrix subregion of the dorsal striatum, we did not see a significant difference in AMPAR/NMDAR ratios in chronic METH treated rats (matrix AMPAR/NMDAR ratios: saline, 1.10116 ± 0.1492; chronic METH, 1.0222 ± 0.054) (Figure 4, Figure 5). When comparing the patch compartments of saline injected animals to chronic METH treated animals, our preliminary findings show no significant difference in the AMPAR/NMDAR ratios, although there was a clear trend toward higher values in chronic METH treated patch neurons (patch AMPAR/NMDAR ratios: saline, 0.9781 ± 0.3826;
chronic METH, $1.7111 \pm 0.3082$) (*Figure 4, Figure 5*). This suggests LTP, although large sample sizes will be required to fully address this apparent difference. When both control groups (saline and naïve rats) were compared to their respective subregions in chronic METH treated rats, there was still no significant difference in the ratios of the respective regions (control AMPAR/NMDAR ratio: matrix – $1.2097 \pm 0.1382$; patch – $1.1598 \pm 0.3388$) (*Figure 6*).
Figure 4. AMPAR/NMDAR ratios in chronic METH treated rats. A) Representative averaged currents for MSNs from the patch and the matrix compartments comparing saline to chronic METH. Matrix MSNs appear unchanged in the two different groups while the patch neurons appear to have a higher AMPAR current in the METH rat compared to the saline-treated rat. B) A representative confocal micrograph showing a neuron located in the patch compartment. The bright red area is stained with mu opioid receptor antibodies while the green area is the neurobiotin filled cell. C) A representative confocal image of a similarly stained MSN, indicating its location in the matrix neuron.
Figure 5. Chronic METH induced synaptic potentiation in patch vs. matrix MSNs.

Summary data showing the comparison of saline neurons from each subgroup to the chronic METH neurons of the subgroups. While the apparent difference observed in chronic METH treated rat dorsolateral MSNs suggests LTP, this difference fell short of statistical significance (n = 4 cells per group).
Figure 6. Chronic METH induced synaptic potentiation in patch vs. matrix MSNs.

Since we did not find any significant difference in the saline vs. naïve groups, we combined the data points to compare all controls to the chronic METH treatment groups, again suggesting LTP in chronic METH treated patch neurons. Furthermore, there was a trend toward slightly lower AMPAR/NMDAR ratio values in chronic METH treated matrix neurons, suggesting a possible LTD, but additional replicated will be needed to determine the consistency of this finding (n = 6 cells per group).
DISCUSSION

Basal AMPAR/NMDAR Ratios Do Not Differ Between Naïve and Saline Controls for Either Striatal Subregion

The behavioral effects of both chronic and acute METH treatment have been well studied and documented (Horner et al, 2010, Horner et al, 2012, Murray et al, 2014). Synaptic plasticity within dopamine neurons of the VTA has been implicated as a mechanism for the formation of addiction when these neurons are repeatedly exposed to drugs of abuse (Kauer and Malenka, 2007). Previous work has shown the dorsal striatum as a relevant brain structure in the creation of addiction (Yager et al, 2015). However, there are currently very few reports that systematically describe the effects of METH on synaptic plasticity within the dorsolateral striatum. Some of these have focused on structural plasticity (Jedynak et al., 2007), or changes in gene expression (Krasnova, et al., 2013), but none have used classical electrophysiology methods to directly explore changes in synaptic strength, particularly in the context of rodent behavior. Therefore, our work first sought to establish initial baseline data focused on the AMPAR/NMDAR ratios for the patch and matrix in both naïve and saline treated rats. We did not find a significant difference in these two groups for the respective areas. However, our sample sizes for these groups were relatively low and will need to be increased in order to validate the findings put forward here.
Chronic METH Treated Rats Show An Increase In Patch Neuron AMPAR/NMDAR Ratios, Suggesting LTP

Our initial findings suggested little to no effect on the AMPAR/NMDAR ratio when rats were treated with acute METH. In the interest of time and to increase the probability of uncovering relevant findings, we therefore transitioned to chronic METH injections soon after the control studies. Although we observed no significant differences in the ratios when compared between the subregions and the study groups, there seems to be evidence of LTP (an increase in the AMPAR/NMDAR ratio) in the patch neurons of chronic METH injected rats. There does not (at least initially) appear to be LTD in the matrix neurons of chronic METH treated rats. However, the sample sizes are still fairly small (n ≤ 4 cells per group) and will need to be increased to determine if the trend is in fact significant. Even with pooling naïve and saline controls (Figure 6), there is still no statistical significance, although the general trend remains, with a slight suggestion of possible LTD in the chronic METH matrix group.
FUTURE DIRECTIONS

Due to issues with data attrition and the acutely toxic effects of METH (rats expiring before data could be acquired), we were not able to collect as much data as we had anticipated in the time allotted. Furthermore, our initial studies suggested that acute treatment with METH that typically induce stereotypic behavior, (Horner et al, 2010; Horner et al, 2012; Murray et al, 2014) had no effect AMPAR/NMDAR ratios. With this in mind, we are currently exploring additional experiments to address our hypotheses. There are two additional ways in which we could study the possible synaptic changes occurring during METH use. One possible approach to studying synaptic plasticity in neurons is to analyze spontaneous EPSCs. LTP and LTD can occur either post-synaptically (as we have studied) or pre-synaptically. Post-synaptic changes manifest in an altered frequency of EPSCs (an increase is LTP and a decrease is LTD), while pre-synaptic causes are seen through altered EPSC amplitudes (an increase for LTP and a decrease for LTD) (Kauer and Malenka, 2007). By using this method, we would be able to determine METH-induced relative differences in excitatory synaptic strength in patch vs. matrix neurons by electrophysiological methods. Previous work dating back to the 1980s has determined differences in firing patterns for patch vs. matrix neurons (Kawaguchi et al, 1989). When depolarized, patch neurons fire at a lower frequency than matrix
neurons, allowing determination of neuron subregions without immunohistochemical staining. However, we were not able to determine patch vs. matrix in this manner during our study due to the cesium based internal solution which we used. Cesium blocks voltage-gated potassium channels, significantly altering action potential waveforms and thus, neuronal firing frequencies, preventing us from using this method to localize MSN subregions in this manner. Future studies of spontaneous EPSCs (instead of AMPAR/NMDAR ratios) will use this method, removing the need for mu opioid receptor staining and increasing the data retention rate.

This study was based on the hypothesis that long-term synaptic plasticity changes occur with either acute or chronic METH use. However, there are also short-term changes in synaptic transmission that can occur in neurons, which may underlie stereotypy with acute METH use. To determine if short-term changes in synaptic function are responsible for METH-induced stereotypy, paired-pulse ratios will be studied. Paired-pulse studies use brief pairs of high-frequency stimulation to force presynaptic neurotransmitter release, and the amplitudes of postsynaptic currents (evoked EPSCs) are then compared as a ratio. These EPSCs are measured to determine if the second pulse current amplitude increases (paired-pulse facilitation) or decreases (paired-pulse depression). This effect decays rapidly after onset and can last from 1 millisecond to tens of milliseconds, and reflects changes in presynaptic neurotransmitter release. Using this approach, we can determine whether short-term changes are occurring in afferent excitatory synaptic
connections with neurons of the dorsal striatum when METH is acutely taken, within the same time period in which stereotypy is observed.
CONCLUSION

Although we were not able to definitively state whether or not METH treatment induces LTP or LTD within the separate subregions of the dorsal striatum, there appears to be a trend towards LTP within the patch compartment. More work is necessary to validate this finding, as well as additional studies to determine if there are other possible synaptic plasticity events occurring in these region in response to METH treatment, and whether or not these changes are responsible for drug-induced behavior in rats.
REFERENCES


