STRIATAL PATCH COMPARTMENT LESIONS REDUCE HABITUAL BEHAVIOR

by

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B.S., Biology, Georgia State University, 2014

A Thesis Submitted to the Graduate Faculty

of 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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ABSTRACT

TERRELL AVONTAE JENRETTE

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Under the direction of KRISTEN A. HORNER, Ph.D.

America spends roughly 600 billion dollars annually on cost related to drug addiction. The majority of current drug addiction research focuses on the rewarding properties of drugs of abuse. The current rate of relapse suggest the need for a greater understanding of habitual drug seeking behavior. The striatum is essential to habit formation and reward association. The striatum is composed of the patch and matrix compartments, and previous studies have shown that enhanced activation of the patch compartment relative to the matrix compartment is related to inflexible behaviors, such as stereotypy. Habitual behaviors are also inflexible in nature, but whether enhanced activation of the patch compartment contributes to habitual behavior is not known. Our experiment analyzed the role of the patch compartment in habit formation, by using a targeted neurotoxin to ablate the neurons of the patch compartment prior to sucrose self-administration. Rats were bilaterally infused in the striatum with the neurotoxin dermorphin-saporin (DERM-SAP) to ablate the neurons of the patch compartment or unconjugated saporin (SAP, as a control) and allowed to recover for eight days. The rats were then trained to self-administer sucrose using a random interval paradigm proven to

generate habitual sucrose consumption. We then associated sucrose consumption with a negative stimulus (LiCl). Subjects were reintroduced to the self-administration chamber and rats that continued to lever press, despite learning to associate sucrose with a negative stimulus, were considered to exhibit habitual behavior.

Our data shows that DERM-SAP pretreatment reduced sucrose self-administration upon reintroduction to the self-administration chamber in animals that received LiCl treatment, indicating that habit formation was attenuated in these animals. DERM-SAP pretreatment also altered c-Fos levels in the sensorimotor cortex, prefrontal cortex, dorsal lateral striatum, and dorsal medial striatum. This data demonstrates that ablation of the patch compartment reduces habitual behavior by reducing activity in the habitual circuit and increasing activity in the goal directed circuit.

INTRODUCTION

In modern society addiction is a well-established and growing problem. According to the National institute on Drug Abuse, the United States spends over 600 billion dollars each year on problems related to substance abuse. Among these cost are healthcare, loss of work productivity, crime, fatalities, and drug treatment therapies. (NIDA et al., 2012). Successful drug treatment therapy could significantly reduce this cost; however most individuals continue to abuse drugs after treatment. This obviously results in greater cost and the loss of many lives. The majority of research regarding drug addiction focuses on circuits that underlie the rewarding properties of drugs of abuse. This information is obviously very valuable but it does not fully explain why drug addiction persists following treatment. There is a very high rate of relapse amongst addicts indicating that more information is required to better understand the mechanisms that underlie habitual drug abuse. A greater knowledge of the neurobiological structures involved in habit formation is necessary for the development of new therapeutic strategies that can restore behavioral control, and complement traditional therapies leading to more effective treatments for addiction.

Previous research has shown that the normal processes that underlie habit formation play a role in the transition from recreational drug use to the compulsive drug seeking behavior that is characteristic of addiction (Yin et al., 2006; Canales et al., 2005; Zapata et al., 2010; Barker et al., 2015). Previous research suggest that in the early stages of habit learning, performance is goal directed and dependent on action-outcome

associations (A-O association), where behavior is sensitive to devaluation of the reward/outcome (Zapata et al., 2010; Chersi et al., 2013; Barker et al., 2015). During the later stages of behavioral learning performance become habitual and relies upon stimulus-response associations (S-R association). Habitual behavior is an autonomic response to a particular sensory input with which the action has become associated (Chersi et al., 2013). Once habitual behavior is established the behavior will continually be produced in response to the stimulus, without concern for the value of the outcome (Canales et al., 2005; Zapata et al., 2010; Chersi et al., 2013). Recent studies have suggested that people addicted to drugs do not exclusively seek drugs for the rewarding properties but also out of habit (Barker et al., 2015). This implies that while drug addiction may initially be goal directed, over time and with repeated exposure, drug seeking behaviors become habitual. Drug abusers essentially over train themselves to the point that behavioral responses are generated by the environment or cues and are independent of the drug's inherent rewarding properties.

Drug addiction hijacks normal habit learning processes. Recent work indicates that habits facilitated by drug abuse, rely on the same neuroanatomical structures that mediate normal habit learning. This provides support for the notion that aberrant habit learning process contribute to addiction (Barker et al., 2015). The striatum plays a significant role in the development of goal directed and habitual reward seeking behaviors (Yin et al., 2006; Balleine et al., 2010; Canales et al., 2005), which is mediated by distinct circuits that originate is the cortex, and transverse the striatum before returning to the cortex (Barker et al., 2015; Balleine et al., 2010). The circuits of interest go through the nucleus accumbens (Everitt et al., 2008) and encode limbic information

related to reward and reinforcement, goal directed behaviors are mediated by circuits connecting the medial prefrontal cortex (mPFC) with the dorsomedial striatum (DMS) and habits are encoded using networks involving the sensorimotor cortex (SMC) and the dorsal lateral striatum (DLS) (Barker et al., 2015; Balleine et al., 2010). The striatum receives dense input from the cortex and some additional dopaminergic input from the midbrain. The midbrain is necessary for the development of habitual behavior (Barker et al., 2015; Chersi et al., 2013). These circuits operate parallel to each other but can also interact via recurrent connections with midbrain dopamine neurons allowing for a ventral-to-dorsal transfer of information (Yin et al., 2006). Limbic information contained in circuits through the nucleus accumbens can influence the sensorimotor network in the dorsal lateral striatum for habit learning (Canales et al., 2005).

Limbic information contained in the circuits of the patch (or striosome) compartment may also influence the sensorimotor networks in the DLS responsible for habit formation. The striatum is composed of both the patch and matrix compartments, which are superimposed on top of each other (Gerfen et al., 1989). The patch and matrix compartment are present in the DLS and DMS. The patch and matrix compartments are neurochemically and anatomically distinct sub-regions of the striatum. The patch compartment contains a high density of mu opioid receptors and receives input from regions of the cortex previously documented to be involved in drug addiction and the formation of S-R association (Canales et al., 2005). The matrix compartment is thought to mediate adaptive behavioral responses based on sensory information. The matrix compartment expresses few mu opioid receptors and receives input from motor and somatosensory cortices (Canales et al., 2005). Previous work has demonstrated that

enhanced activation of the patch compartment results in repetitive and inflexible behaviors. Previous research has also shown that targeted destruction of the patch compartment prevents psychostimulant-induced repetitive behavior (Canal et al., 2006; Horner et al., 2015). Enhanced activation of the patch compartment in the DLS could represent an enhancement in the rewarding and motivational aspects of behavior. In the context of the sensorimotor region of striatum, the focus of the behavior is narrowed, while at the same time the tendency to repeat the behavior is increased and becomes more inflexible in nature (Canales & Graybiel et al., 2000). We believe that preferential activation of the patch compartment plays an active role in the transition from casual drug use, which is goal directed to the habitual use of drugs seen in addiction. However, given that stereotypy and habitual behavior both lead to rigid and inflexible behavior, it is possible that enhanced activation of the patch compartment in the DLS plays a major role in habit learning, in general. In support of this hypothesis, our studies indicate that ablation of the patch compartment neurons in the DLS attenuates habitual sucrose consumption, which implies that enhanced activation of the patch compartment may go beyond addiction and could be generalized to all habit learning.

In addition to the patch compartment's role in the development of habitual behaviors, the patch compartment also contributes to reward processes. Previous work has shown that animals will continually self-stimulate if an electrode is placed in the patch compartment (White & Hiroi et al., 1998), and recent data from our laboratory has shown that reduced activation of the patch compartment is associated with a decrease in the rewarding effects of methamphetamine (METH) (Horner et al., 2017). The neurons of the patch compartment receive input from the limbic structures that mediate affective

behaviors (mood, motivation, and emotion), which reasonably suggest that the patch compartment may have reward related functions. Specifically the patch compartment receives input from the medial prefrontal cortex, which plays a role in goal directed behavior. Previous data suggest that the patch compartment may have reward-association functions, but it is not currently known whether patch-enhanced activity in the striatum occurs with drug-related reward.

The mechanisms by which patch based circuits become preferentially enhanced following chronic psychostimulant treatment or in the context of repetitive and inflexible behaviors are poorly understood. It has been suggested that changes in glutamate-mediated neural plasticity, such as long-term potentiation and/or long term depression in the neurons of the striatum contribute to the function of the patch based circuits. Enhanced activation of the patch compartment following psychostimulant treatment has been associated with decreased gene expression in the matrix compartment. This suggests that repeated exposure to psychostimulants may induce LTD in the neurons of the matrix (Canales & Graybiel et al., 2000; Graybiel, et al., 2000). The sensorimotor cortex, which projects mainly to the matrix compartment is strongly activated by psychostimulants, and a progressive depression of glutamatergic input from the cortex could account for the down-regulation of gene expression in the matrix compartment (Canales & Graybiel et al., 2000; Graybiel et al., 2000; O'Dell & Marshall et al., 2000). Repeated psychostimulant exposure may lead to long-lasting changes in neural plasticity within the matrix compartment and could contribute to reward and reinforcement. The matrix compartment has been documented to play a role in the maintenance of normal adaptive

sensorimotor behavior; which is beneficial in a constantly changing environment (Brown et al., 2002).

The research discussed above suggest that enhanced activation of patch-based circuits, relative to matrix-based circuits may contribute to reward processes and could participate in the transition from goal-oriented to habitual behaviors. We hypothesize that ablation of the patch compartment will reduce habitual behavior (and increase goal directed behavior). We also hypothesize that ablation of the patch compartment will alter the flow of information through the basal ganglia circuits (goal directed and habitual circuits).

MATERIALS AND METHODS

Animals

Male Sprague- Dawley rats (Charles River, Durham, NC), weighing between 290-400g were used for the experiment. Prior to surgery rats were housed in groups of four in plastic containers within a temperature controlled room. Rats were given free access to food/water, and were placed on a 14:10 hour light/dark cycle. All animal care and experimental manipulations were approved by the Institutional Animal Care and Use Committee (IACUC) at Mercer University School of Medicine and were in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Measures were taken to minimize suffering and the number of animals required to complete this experiment.

Drugs and Chemicals

Ketamine hydrochloride and xylazine hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO, USA). Both ketamine and xylazine doses were dissolved in normal saline. All drugs were given in a volume of 1 ml/kg. Dermorphin-saporin (DERM-SAP) and unconjugated saporin (SAP) were dissolved in artificial cerebrospinal fluid (aCSF: 144 mM NaCl, 2.68 mM KCl,1.6 mM CaCl2, 2.6 mM MgCl2, 0.4 mM KH2PO4, pH 7.2).

DERM-SAP Infusion

Ketamine and xylazine were used to anesthetize the rats. Anesthetized rats were held into positon during surgery using a stereotaxic frame (Stoelting Company, Wood Dale, IL, USA). Two holes were drilled into the skull of each rat. A 30-gauge needle was introduced to both holes in the skull to access the striatum. A total volume of 2 µl DERM-SAP or SAP was delivered bilaterally to the rostral striatum. Half of the rats received DERM-SAP and the other half received SAP. The location of the striatum was coordinated using bregma as a marker (.7 mm anterior, 2.6 mm lateral, -5.0 mm ventral; Paxinos and Watson 2005). DERM-SAP or SAP (Tokuno et al. 2002) was administered at a rate of 0.5 µl/min. The needle was left in place for 5 minutes following the infusion and slowly removed to minimize fluid backflow. Only animals whose infusions were placed properly in the striatum were included in subsequent analyses.

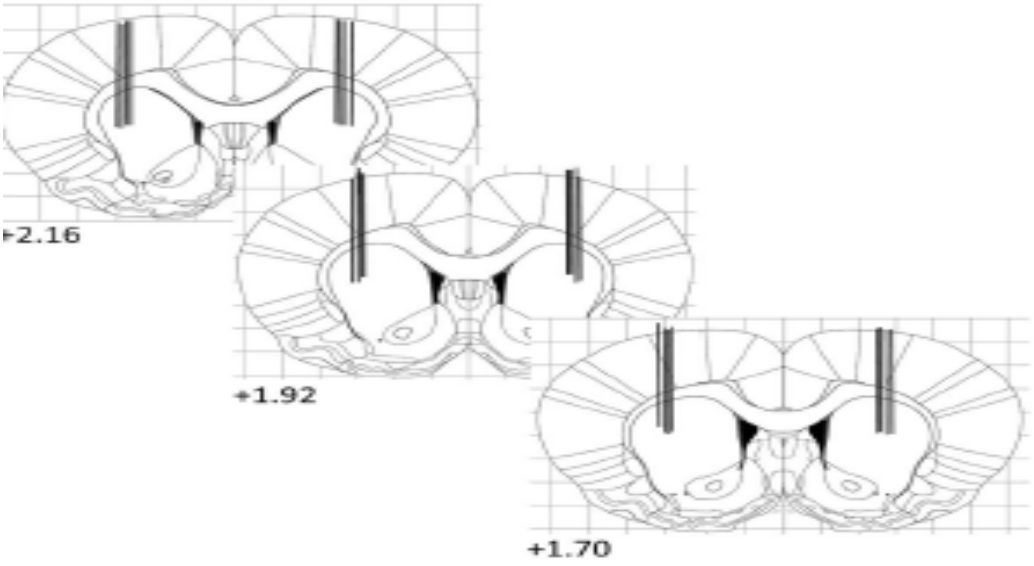

Fig. 1 Diagram of properly placed micro infusion cannulae tips. Cannulae tips were placed using bregma as a marker (.7 mm anterior, 2.6 mm lateral, -5.0 mm ventral).

Chamber Set-up

Rats were adequately trained to lever press. All lever training took place in operant chambers within sound and light attenuating boxes (Medical Associate Inc., St Albans, VT). Our lab used four operant chambers and each chamber contained two retractable levers on either side of a recessed magazine trough. When the left lever is pressed appropriately, 0.08 ml of 20% sucrose solution was delivered from a magazine into a liquid trough.

S-R Associated Learning Using a Random Interval Paradigm

Prior to sucrose training rats were placed on a food restricted diet (Approximately 4 pellets per day) until rats reached 80% body weight. Rats repeated 30 minute magazine training sessions twice daily for two consecutive days, without levers present. On the first day of training sucrose was delivered with 100% probability every 15 seconds. On the second day of magazine training sucrose was delivered with 100% probability every 60 seconds. On days three and four rats received continuous reinforcement (FR1 schedule). The FR1 schedule delivered sucrose with 100% probability with every individual lever press. On day five through day ten the rats were delivered sucrose at random intervals (RI15, RI30, and RI60). Days five and six, sucrose was delivered on average every 15 seconds, with quantities determined by lever pressing. Days seven and eight, sucrose was delivered on average every 30 seconds, with quantities determined by lever pressing. Days nine and ten sucrose was delivered on average every 60 seconds, with quantities determined by lever pressing. This particular series of random intervals was chosen because it was proven to promote habit formation (Yin and Knowlton, 2006; Son, et al., 2011). Rats were subjected to outcome devaluation from day 11 to day 13. During

outcome devaluation all rats were given 30 minute access to a bottle containing 20 % sucrose solution. The amount of sucrose consumed by each rat was recorded daily. Immediately following sucrose access half of the rats received a daily dose of LiCl and the other half received a daily dose of saline. The extinction phase occurred on day 14. Rats were placed in operant chambers for 10 minutes. Lever pressing was recorded but did not result in sucrose administration.

Tissue Processing and C-Fos Immunohistochemistry

After experimentation rats were sacrificed via exposure to CO_2 followed by decapitation. Rat brains were frozen in isopentane on dry ice and stored at -80 C until they were cut into 16-µm sections through the frontal cortex, and striatum on a cryostat (Minotome Plus, Triangle Biomedical Sciences, Durham, NC, USA). These specific tissue slices were mounted onto slides and frozen for later use.

Sections from the prefrontal cortex and striatum were thawed and circled with a PAP pen. Slides were washed 3 times in PBS, with each wash lasting 5 minutes in duration. Tissues were fixed onto slides with 4 % paraformaldehyde and then rinsed three times in PBS. Slides were then blocked for 1 hour at room temperature with 4% normal donkey serum (NDS), 4% dry milk powder, 0.2% triton-x-100 and avidin block. Slides were then covered with a polyclonal antibody for c- Fos diluted 1:1,000 in 0.3 % TX/0.1 M PBS and incubated overnight at 4 C. The next day the slides were washed 3 times with PBS and then incubated for 2 hours with horse anti-rabbit IgG antiserum (Vector laboratories) in PBS. The slides were washed 3 times in PBS, incubated 1 hour in ABC solution (Elite ABC kit, Vector Laboratories) and washed three more times in PBS. Slides were covered in a DAB –Nickel solution for approximately 2 minutes and then

washed again with PBS, followed by a 10 minute wash in deionized water. Lastly slides were dehydrated by a series of alcohols and cover slipped out of xylene.

Image Analysis

To confirm c-Fos particle concentration in the dorsal lateral striatum, dorsal medial striatum, prefrontal cortex, and sensorimotor cortex images were captured with a VistaVision microscope connected to a video camera (CCD IEEE-1394, Scion Corporation, Frederick, MD, USA), using a 4X objective. On the striatum, prefrontal cortex, and secondary motor cortex images three to four random c-Fos particles were outlined using ImageJ software (National Institutes of Health; http://rsb.info.nih.gov/ij). The smallest particles from the first image were recorded. This was repeated on four additional randomly selected slides of each tissue type and averaged to determine the lower limit required to perform an accurate particle analysis.

The number of c-Fos particles that exceed the threshold size was determined using the particle analysis option in ImageJ. The threshold size was adjusted such that background staining was eliminated and the number of c-Fos represented all particles above the size threshold. To determine if DERM–SAP treatment altered the activity level of both the dorsal medial and dorsal lateral striatum, the total number of c-Fos particles highlighted by thresholding in both the left and right hemispheres was averaged for each animal. The number of particles in the sensorimotor cortex, prefrontal cortex, dorsal medial striatum and dorsal lateral striatum (Figure 2) were analyzed over a 500 x 500 pixel area for sensorimotor cortex and prefrontal cortex, and a 1024 x 768 pixel area for dorsomedial and dorsolateral striatum.

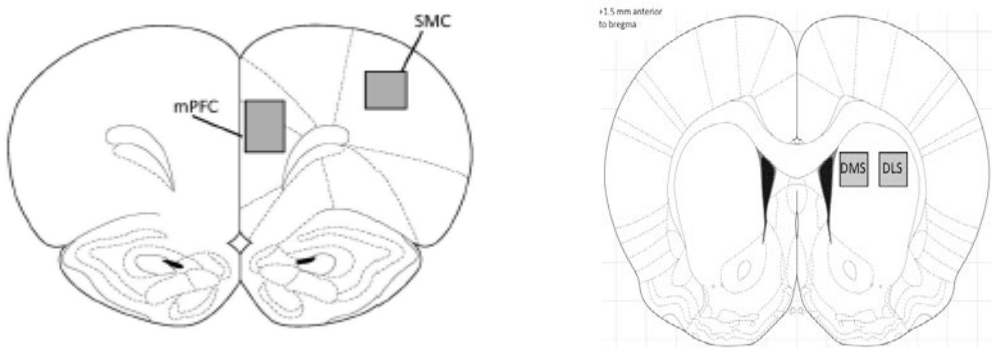

Fig. 2 this schematic details the sub regions of the prefrontal cortex and striatum that were used for the quantitative analyzes of c-Fos expression. The regions used for c-Fos immunoreactivity are highlighted in gray (medial prefrontal cortex, sensorimotor cortex, dorsal lateral striatum and dorsal medial striatum).

Statistical Analysis

A two way ANOVA was used to determine the correlation between the effects of DERM-SAP pretreatment and taste aversion treatment on the number of c-Fos particles in the prefrontal cortex, sensorimotor cortex, and striatum (lateral medial striatum and dorsal medial striatum). A post-hoc analysis was used to compare all four groups (DERM-SAP/LiCl, DERM-SAP/saline, SAP/LiCl, SAP/saline), two at time to determine the exact nature of significance. Behavioral data was also analyzed using a two way ANOVA comparing the effect of DERM-SAP pretreatment and taste aversion treatment on sucrose consumption. Behavioral data was subjected to a similar post-hoc analyses as the c-Fos data.

RESULTS

To evaluate the effect of ~~the~~ DERM-SAP on habit formation via S-R learning, rats were trained using an RI schedule of reinforcement. RI schedules of reinforcements are proven to promote the formation of S-R association (Dickinson, 1985). S-R association was maintained throughout the RI reinforcement schedule (Figure 3). All rats regardless of pretreatment learned to use the lever press to administer sucrose. When the random interval phase was complete a statistical analysis (one-way ANOVA) was performed to rule out any difference between lesioned and unlesioned animals. This analysis reported that there was no significant difference in the amount of lever pressing between SAP and DERM-SAP animals during the FR1 training ($p>0.05$), RI15 training ($p>0.05$), RI30 training ($p>0.05$) or RI60 training ($p>0.05$). In order to determine whether the instrumental behavior reflected an underlying S-R association, it was necessary to introduce a conditioned taste aversion (CTA) phase. During the CTA phase half of the animals received saline and half received LiCl. Rats subjected to saline treatment did not exhibit significant differences in sucrose consumption (Figure 3). This finding is consistent with previous literature (Yin et al, 2004).

Rats subjected to LiCl decreased sucrose consumption over the course of the three days (Figure 4). A two way ANOVA revealed a significant main effect of devaluation ($F_{1,150=264}$,$p<.0001$), a main effect of each day ($F_{1,150}=19.63$, $p<.0001$), and significant devaluation x day interaction ($F_{1,150}=58.16$, $p<0001$).

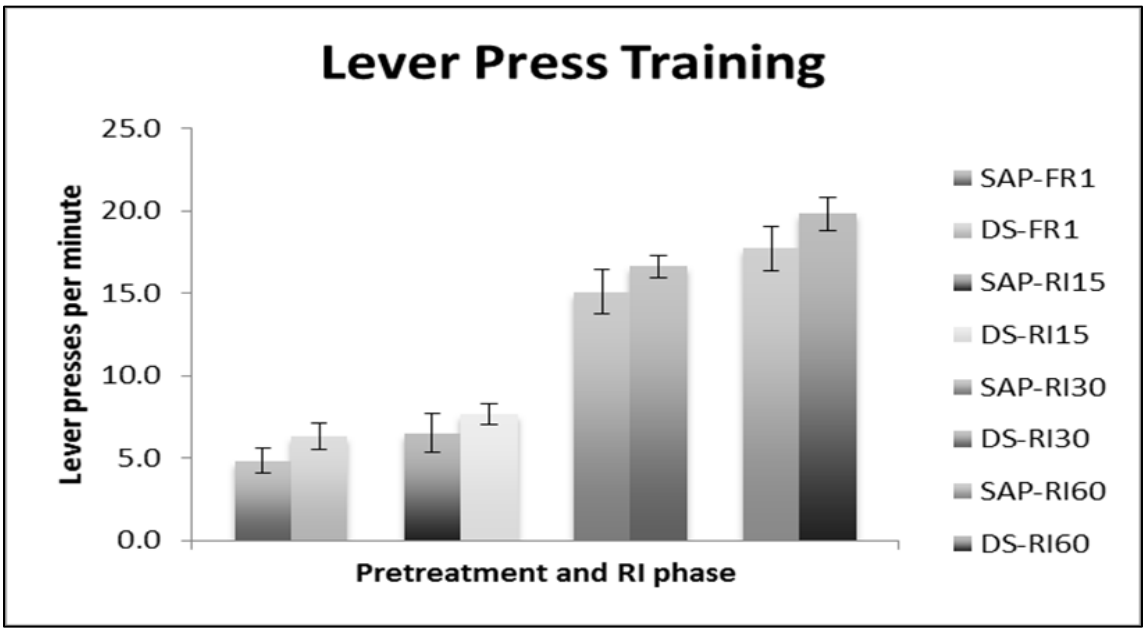

Fig. 3 This graph compares lever pressing for animals pretreated with DERM-SAP or saline. There was no significant difference in lever pressing between DERM-SAP and saline treated animals.

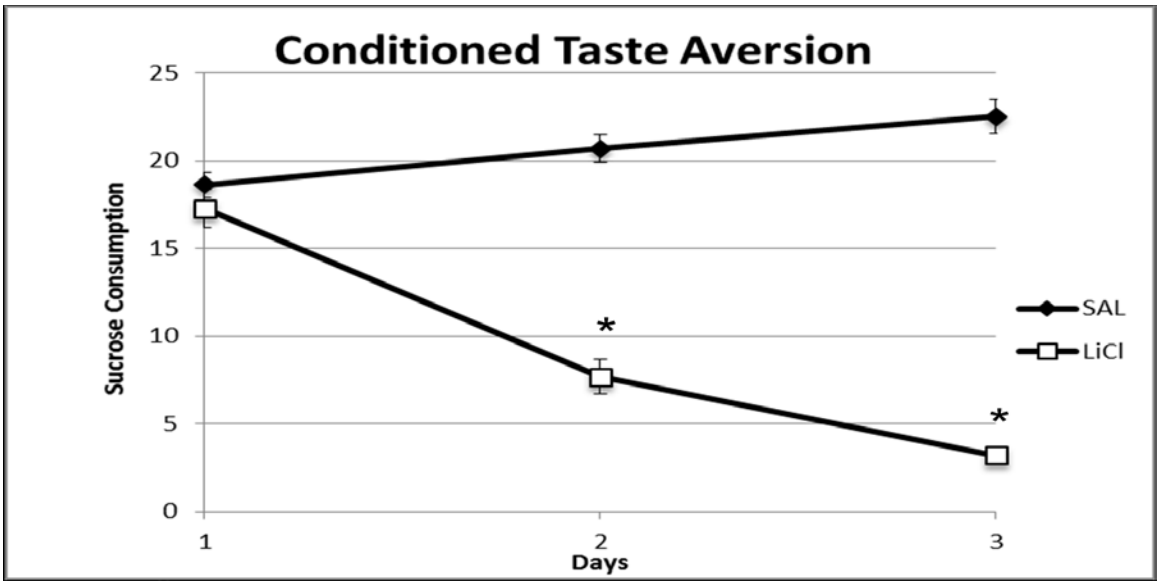

Fig. 4 Effects of LiCl on stimulus- response (S-R) associated instrumental learning. LiCl conditioned taste aversion took place over 3 days. The data expressed is the mean mg (±SEM) of sucrose solution consumed in the home cage over the course of 30 minutes. Animals were then injected with either saline or LiCl. There was a significant difference in sucrose consumption when comparing saline and LiCl treated animals.

Data pertaining to sucrose consumption from RI60 and the extinction phase were compared and analyzed to determine the extent of outcome devaluation (Figure 5). A two way ANOVA revealed a main effect of pretreatment ($F_{1,25}$=6.1,p=.0207), devaluation ($F_{1,25}$=6.1, p=.0207), and pretreatment x devaluation interaction ($F_{1,25}$=5.8, p=0.0238). A post-hoc analysis revealed that lesioned animals exhibited significantly greater devaluation of sucrose following taste aversion than unlesioned animals ($p<.05$). There was significant devaluation of sucrose in lesioned animals that received LiCl compared to lesioned animals that received saline ($p<.05$). Unlesioned animals did not significantly differ in lever presses per minute regardless of treatment ($p>.05$). There was no significant devaluation of sucrose between lesioned animals treated with saline and unlesioned animals treated with saline ($p>.05$).

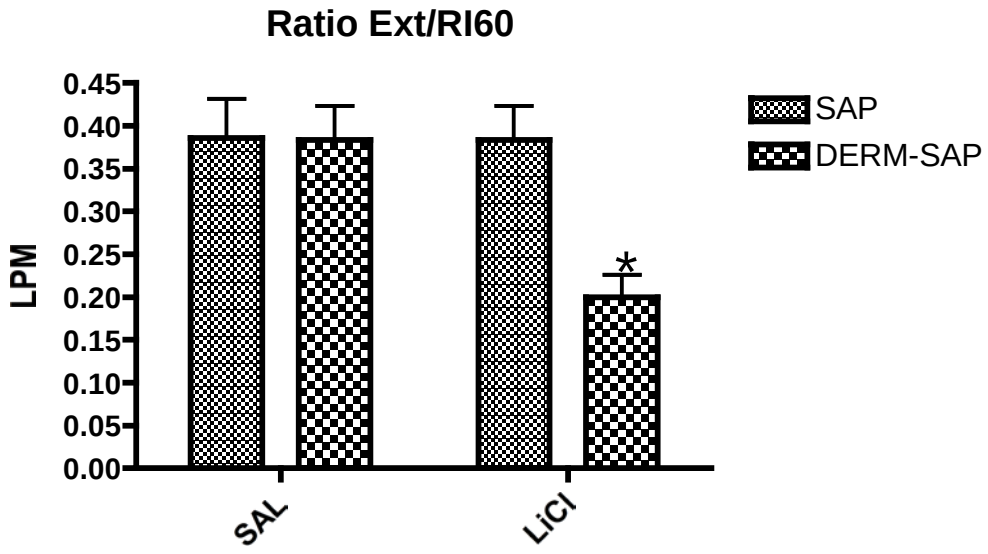

Fig. 5 Effect of LiCl induced taste aversion on extinction responding in SAP vs DERMSAP treated animals trained on RI schedule of reinforcement. This graph documents the ratio of lever pressing when comparing the 10 minute extinction testing to the lever pressing observed during the RI60 phase of this experiment.

Effects of DERM–SAP Pretreatment on C-Fos Immunoreactivity

in the Dorsal Medial Striatum

A two way ANOVA revealed a main effect of DERM-SAP pretreatment in the dorsal medial striatum ($F_{1,13}$= 4.688, $p<0.0496$), LiCl treatment ($F_{1,13}$=6.598, $p<.0234$) and pretreatment x treatment interaction ($F_{1,13}$=7.930, $p<.0146$). Post-hoc analyses revealed that lesioned animals treated with LiCl had significantly increased c-Fos expression compared to lesioned rats treated with saline ($p<.05$). Unlesioned animals treated with LiCl exhibited significantly less c-Fos expression when compared to DERM-SAP pretreated animals exposed to LiCl ($p<.05$). There was no significant difference in c-Fos expression between lesioned rats treated with saline and unlesioned rats treated with saline ($p>.05$). Unlesioned animals did not significantly differ in c-Fos expression regardless of treatment (LiCl/saline)($p>.05$).

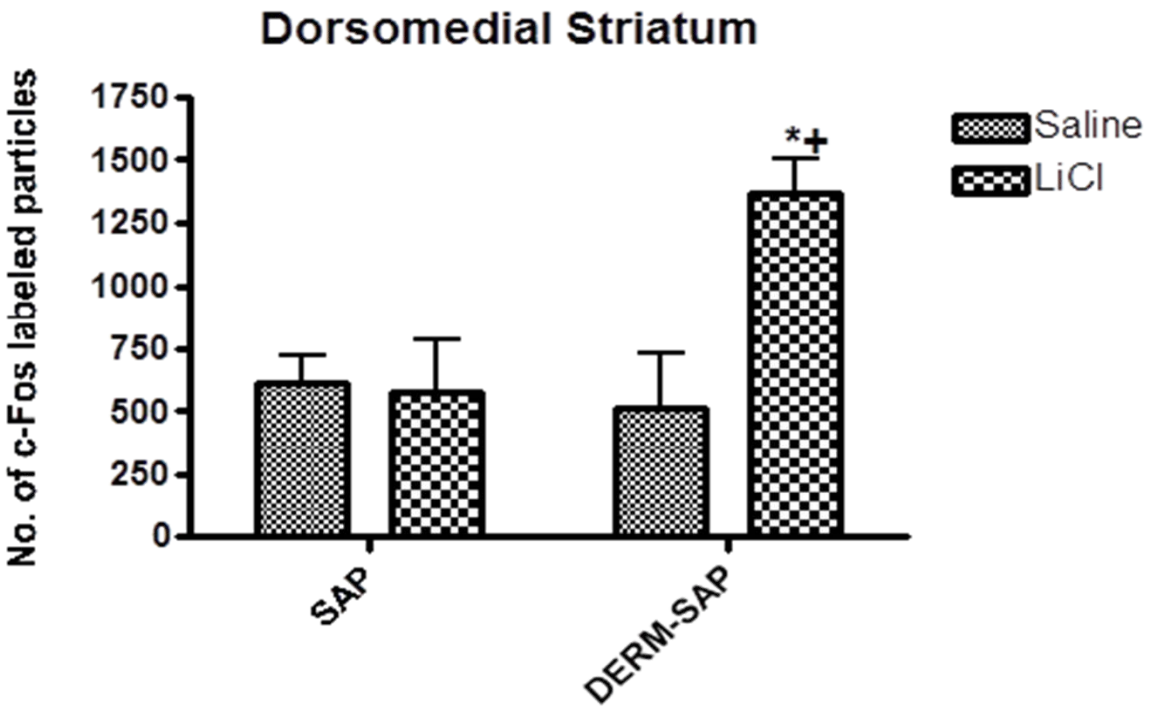

Fig. 6 Effects of DERM-SAP pretreatment on c-Fos expression in the dorsal striatum. Quantitative analysis of c-Fos immunoreactivity in the dorsal medial striatum. The data presented in the graph are the number of c-Fos particles gathered from a 1024 x 768 pixel area on the dorsal medial striatum. The graph also compares the effect of LiCl on the c-Fos expression of lesioned and unlesioned animals.

Effect of DERM-SAP Pretreatment on C-Fos Immunoreactivity

in the Dorsal Lateral Striatum

A two way ANOVA of the c-Fos particle concentration revealed a main effect of DERM-SAP pretreatment in the dorsal lateral striatum ($f_{1\text{-}13}$= 7.500, p< .0169), LiCl treatment ($f_{1\text{-}13}$= 10.42, p<.0066), and significant treatment x pretreatment interaction (f1-13=18.05, p<.0169). Post-hoc analyses revealed that DERM-SAP animals pretreated with LiCl expressed significantly less c-Fos than DERM-SAP pretreated animals treated with saline (p<.05). There was not a significant difference in the expression of c-Fos between lesioned and unlesioned animals treated with saline (p>.05).

There was no significant difference in c-Fos expression in animals pretreated with SAP regardless of treatment ($p>.05$). There was significantly more c-Fos particle expression in unlesioned animals treated with LiCl compared to lesioned animals treated with LiCl ($p<.05$).

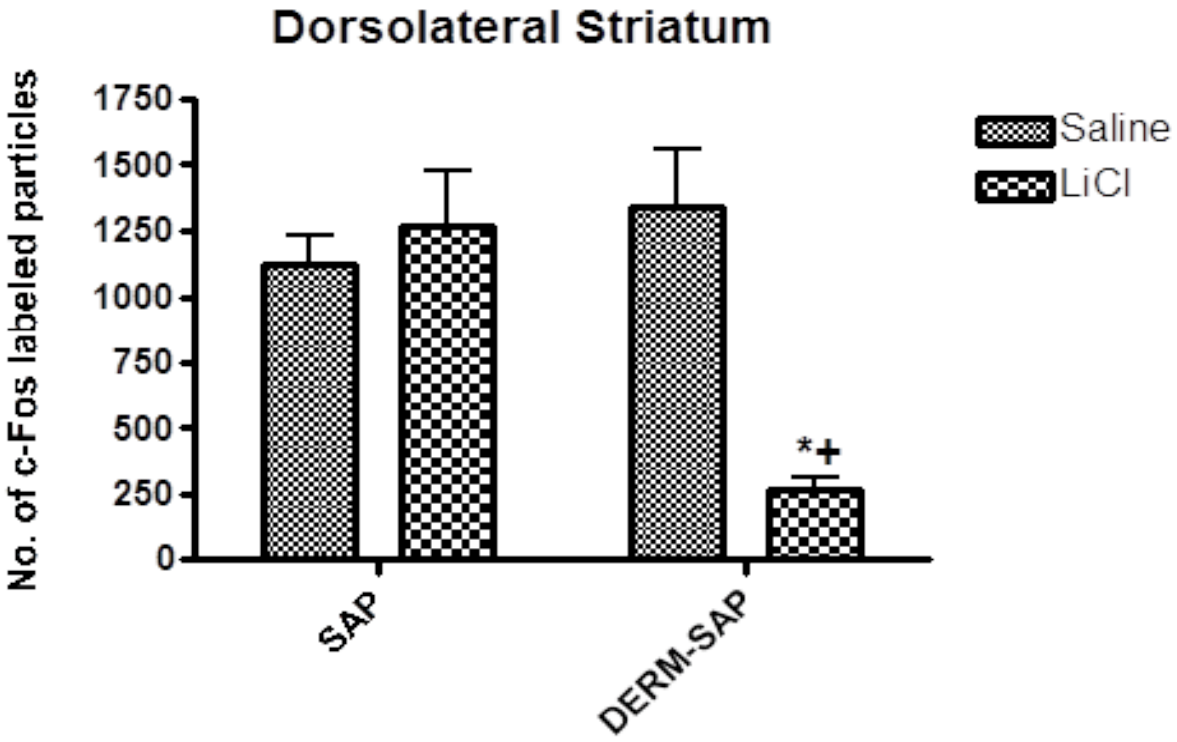

Fig. 7 Effects of DERM-SAP pretreatment on c-Fos expression in the dorsal lateral striatum. Quantitative analysis of c-Fos immunoreactivity in the dorsal lateral striatum. The data presented in the graph is the number of c-Fos particles gathered from a 1024 x 768 pixel area on the dorsal lateral striatum. The graph also compares the effect of LiCl on the c-Fos expression of lesioned and unlesioned animals.

Effect of DERM-SAP Pretreatment on C-Fos Immunoreactivity in the Prefrontal Cortex

A two way ANOVA of c-Fos expression revealed a main effect of DERM-SAP pretreatment in the prefrontal cortex ($f_{1,14}$=8.697, p=.0106), LiCl pretreatment ($F_{1,14}$=.4.985, p=.0424) and significant pretreatment x treatment interaction ($F_{1,14}$=21.04, p=.0004). A post-hoc analysis revealed a significant difference in c-Fos expression in animals pretreated with DERM-SAP and treated with LiCl versus animals pretreated with DERM-SAP and treated with saline (P<.05). There was significantly more c-Fos expressed in animals pretreated with DERM-SAP and treated with LiCl vs animals pretreated with SAP and treated with LiCl (p<.05). There was no significant difference between animals pretreated with SAP and treated with saline versus animals pretreated with SAP and treated with LiCl (P>.05). There was also no significant difference between animals pretreated with SAP and treated with saline when compared to animals pretreated with DERM-SAP and treated with saline (P>.05).

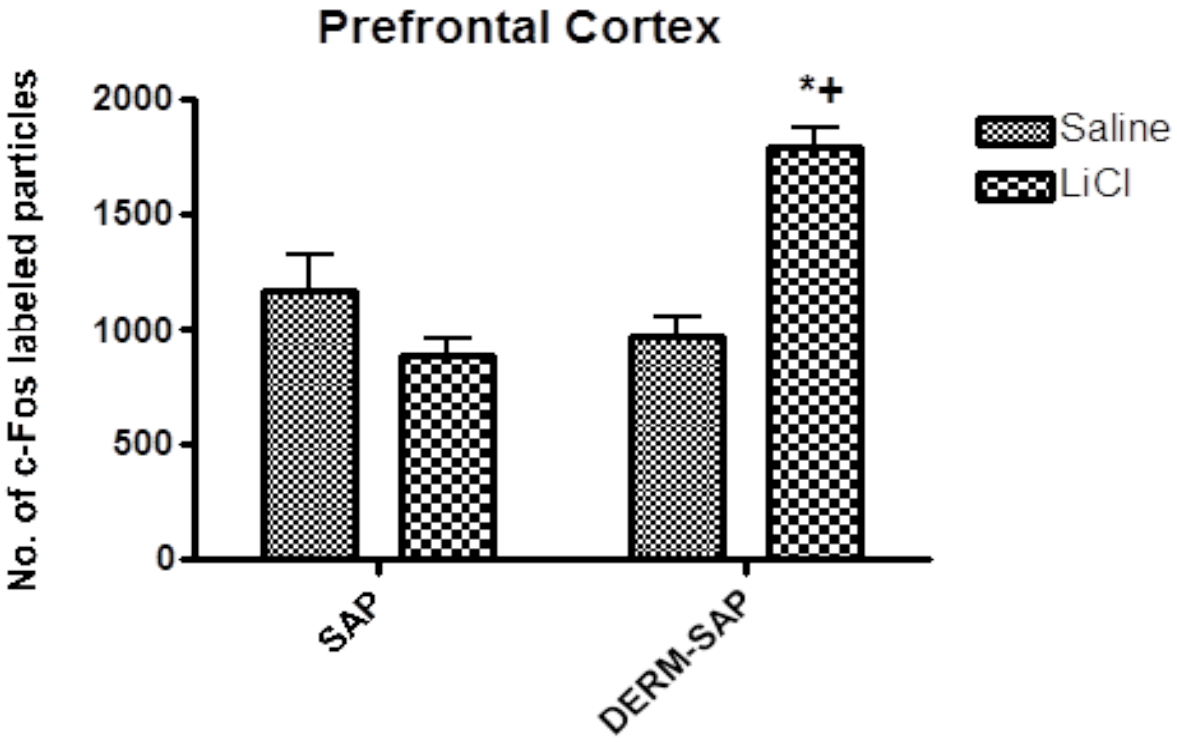

Fig. 8 Effects of DERM-SAP pretreatment on c-Fos expression in the prefrontal cortex. Quantitative analysis of c-Fos immunoreactivity in the prefrontal cortex. The data presented in the graph is the number of c-Fos particles quantified from a 500 x 500 pixel area of the prefrontal cortex. The graph also compares the effect of LiCl on the c-Fos expression of lesioned and unlesioned animals.

Effect of DERM-SAP Pretreatment on C-Fos Immunoreactivity

in the Sensorimotor Cortex

A two way ANOVA of c-Fos expression revealed a main effect of DERM-SAP pretreatment in the secondary motor cortex ($f_{1,19}$=6.413, p=.0203), LiCl pretreatment ($F_{1,19}$=7.374, p=.0137) and significant pretreatment x treatment interaction ($F_{1,19}$=16.54, p=.0007). A post-hoc analysis revealed a significant difference in c-Fos expression in lesioned animals treated with LiCl versus lesioned animals treated with saline (P<.05).

There was a significant difference in c-Fos expression in unlesioned animals treated with LiCl when compared to lesioned animals treated with LiCl ($p<.05$). There was not a significant difference in c-Fos expression between unlesioned animals treated with saline versus lesioned animals treated with saline ($P>.05$). There was also no significant difference between SAP pretreated animals regardless of treatment ($P>.05$).

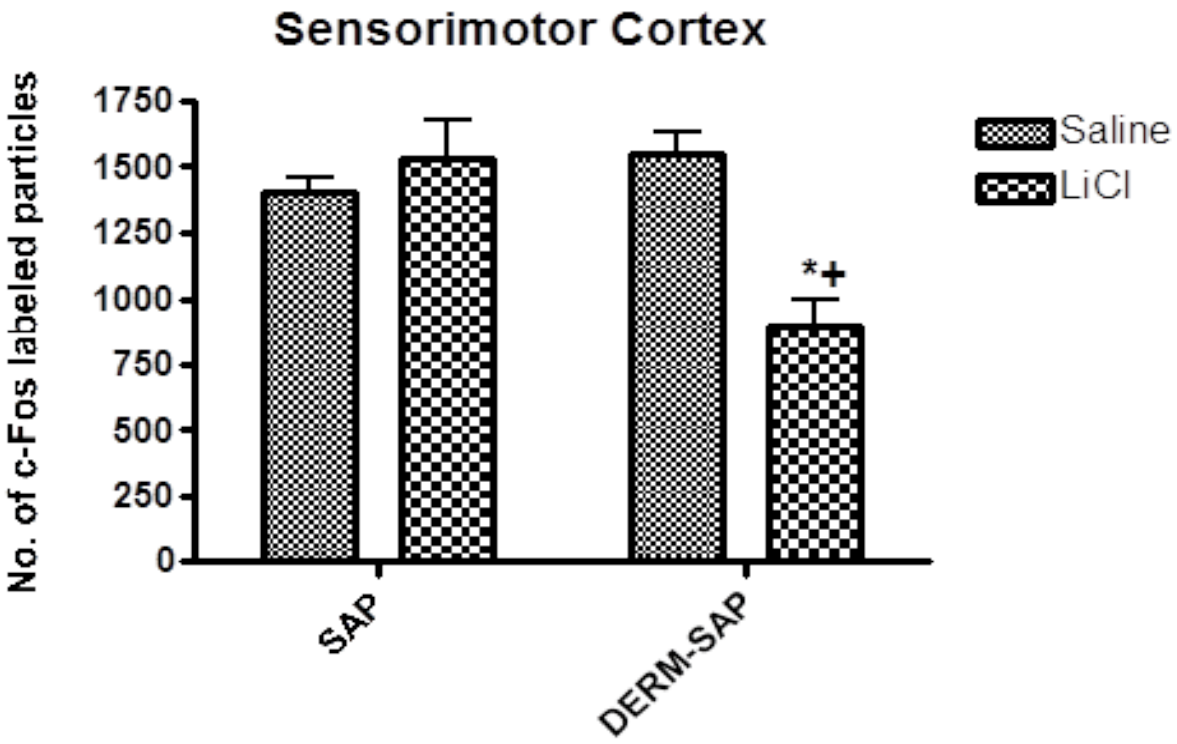

Fig. 9 Effects of DERM-SAP pretreatment on c-Fos expression in the sensorimotor cortex. Quantitative analysis of c-Fos immunoreactivity in the sensorimotor cortex. The data presented in the graph is the number of c-Fos particles quantified from a 500 x 500 pixel area of the sensorimotor cortex. The graph also compares the effect of LiCl on the c-Fos expression of lesioned and unlesioned animals.

DISCUSSION

Effect of Lesioning Animals on Lever Presses Per Minute

The initial goal of this study was to determine if eliminating the patch compartment reduced the occurrence of habitual behavior. Previous research has shown a correlation between enhanced activation of the patch compartment and inflexible or repetitive behaviors in animals. A habit could also be described as repetitive or inflexible so we concluded that ablation of the patch compartment should also reduce habitual behavior. The secondary goal of this study was to determine if ablation of the patch compartment would alter the flow of information through the basal ganglia circuits. The basal ganglia circuits of interest are the goal directed behavior circuit and the habitual behavior circuit.

In this study we lesioned the patch compartment in animals using DERM-SAP. Previous studies that attempted to reduce habitual behavior by removing the dorsal lateral striatum entirely noted that some animals were unable to lever press following surgery (Yin et al., 2006). We hoped to avoid this occurrence by specifically targeting the patch compartment instead of the entire dorsal lateral striatum. When we compared lever press training data (FR1, RI-15, RI-30, and RI-60) from lesioned animals and unlesioned animals there was no significant difference between lever presses per minute. These results confirmed that DERM-SAP pretreatment does not hinder the ability of rats to learn to lever press.

Following RI-60 lever training rats completed the conditioned taste aversion portion of the experiment. There was significantly less sucrose consumed by animals treated with LiCl when compared to animals treated with saline. This proved that sucrose was devalued to all rats (DERM-SAP and SAP pretreated) that received LiCl. Following conditioned taste aversion we placed the rats back in the self-administration chambers to determine if they were willing to lever press for the previously devalued sucrose. If rats that were pretreated with DERM-SAP and treated with LiCl continued to lever press at the same rate that would imply that the behavior was habitual in nature. If rats that were pretreated with DERM-SAP and treated with LiCl significantly decreased the rate of lever pressing that would suggest a reduction in habitual behavior and an increase in goal directed behavior. Actual data from this experiment showed a 40-50% devaluation of sucrose when comparing lesioned animals treated with LiCl to all other control groups (lesioned animals treated with saline, unlesioned animals treated with LiCl, and unlesioned animals treated with saline). Habitual behavior occurs without concern for the value of the reward so animals exhibiting habitual behavior would not devalue sucrose in response to its association with a negative stimulus. The DERM-SAP pretreated animals in this study however were more flexible and able to adjust their behavior in response to an adverse consequence of sucrose consumption. This provides behavioral evidence that lesioning of the patch compartment in the dorsal lateral striatum significantly reduces habitual behavior.

Goal Directed Circuits and S-R Habit Directed Circuits

When we compared c-Fos expression data from the prefrontal cortex, sensorimotor cortex, dorsal medial striatum and dorsal lateral striatum certain patterns were observed. C-Fos particles are used as a measure of neuronal activity. The varying expression of c-Fos indicates which structures were the most active during the last phase of the experiment. Prefrontal cortex and dorsal medial striatum are neuroanatomical structures associated with the goal directed circuit. Sensorimotor cortex and dorsal lateral striatum are neuroanatomical structures of the habitual circuit.

Lesioned animals treated with LiCl had more c-Fos particles in the prefrontal cortex and dorsal medal striatum compared to unlesioned animals treated with LiCl. These structures are a part of the goal directed circuit and are associated with goal directed behavior. Increased neuronal activity within these structures corresponds with the behavioral data for animals pretreated with DERM-SAP and treated with LiCl. This c-Fos data demonstrates an increased activity in the goal directed circuit. Both c-Fos and behavioral data suggest that animals pretreated with DERM-SAP and treated with LiCl exhibit goal directed behavior. In nature animals use goal directed behavior to react appropriately to the environment or adjust actions to produce the more favorable outcome.

Patch compartment lesioned animals treated with LiCl expressed less c-Fos in the dorsal lateral striatum and the sensorimotor cortex than unlesioned animals treated with LiCl or saline. This suggests that removal of the patch compartment from the dorsal lateral striatum caused the decrease in neuronal activity levels within these two structures. This data corresponds perfectly with behavioral data for lesioned animals

treated with LiCl; which exhibited significant sucrose devaluation or reduction of habit formation. Behavioral data for unlesioned animals treated with LiCl exhibited reduced devaluation of sucrose indicating the presence of habitual behavior. This simply demonstrates that it is the pretreatment with DERM-SAP or ablation of the patch compartment that causes the change in behavioral performance and not LiCl.

One unexpected finding that we observed in the dorsal lateral striatum and the sensorimotor cortex was that there was significant expression of c-Fos in animals that were pretreated with DERM-SAP and treated with saline. These animals never associated sucrose with a negative stimulus and thus never devalued sucrose. Coming into the study, we believed that simply ablating the patch compartment of the dorsal lateral striatum would reduce the occurrence of habitual behavior. The data however suggest that patch compartment plays a role in producing habitual behavior despite the presence of a negative stimulus or a devalued reward. The DERM-SAP pretreated and saline treated animals had the lowest test subject numbers (N=3) so this finding may be inaccurate due to the small sample size. Additional research is required to test the validity of results gained from the DERM-SAP pretreated, saline treated animals.

In summary, our findings are the first to demonstrate the role of the patch compartment in habitual learning. Our data also indicates that ablation of the patch compartments disrupts the flow of information through basal ganglia circuits, such that activity in the circuits that mediate habitual behaviors is decreased, while activity in the circuits that mediate goal-directed behaviors is increased. The data suggest that ablation of the patch compartment results in greater behavioral flexibility as reflected in the increased activity in goal-directed circuits.

Further Studies

In the future we would like to further investigate the role of the limbic system in habit formation. The patch compartment is limbic in nature because it receives input from the prefrontal cortex. This may suggest that there is limbic influence over habitual behavior. The limbic system also provides input to the matrix compartments via the prefrontal cortex. We are curious about how the limbic system can provide input to two functionally opposite circuits. Further research is required to determine the extent to which the limbic system contributes to goal directed and habitual behavior. Another topic of interest in this study is understanding the mechanisms that allow the patch compartment to exert control over habitual behavior. Our research demonstrated that the patch compartment certainly plays a role in habit formation but it does not explain the mechanism. A greater understanding of the patch compartments influence over habitual behavior may provide a target for treatment therapies. These treatment therapies will not just be limited to addiction but may also have ramifications for other behavioral disorders characterized by inflexible behaviors such as Tourette syndrome, autism, and obsessive compulsive disorder.

REFERENCES

1. Balleine, B.W. & O'Doherty, J.P. (2010) Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. Neuropsychopharmacology, 35, 48-69.

2. Barker, J.M., Corbit, L.H., Robinson, D.L., Gremel, C.M., Gonzales, R.A. & Chandler, L.J. (2015) Corticostriatal circuitry and habitual ethanol seeking. Alcohol, 49, 817-824.

3. Brown, L.L., Feldman, S.M., Smith, D.M., Cavanaugh, J.R., Ackermann, R.F. & Graybiel, A.M. (2002) Differential metabolic activity in the striosome and matrix compartments of the rat striatum during natural behaviors. J Neurosc, 22, 305-314.

4. Canales, J.J. (2005) Stimulant-induced adaptations in neostriatal matrix and striosome systems: transiting from instrumental responding to habitual behavior in drug addiction. Neurobiology Learn Mem, 83, 93-103.

5. Canales, J.J. & Graybiel, A.M. (2000) A measure of striatal function predicts motor stereotypy. Nat Neurosc, 3, 377-383.

6. Chersi, F., Mirolli, M., Pezzulo, G. & Baldassarre, G. (2013) A spiking neuron model of the cortico-basal ganglia circuits for goal-directed and habitual action learning. Neural Netw, 41, 212-224.

7. Corbett, D., Laferriere, A. & Milner, P.M. (1982) Plasticity of the medial prefrontal cortex: facilitated acquisition of intracranial self-stimulation by pretraining stimulation. Physiology & behavior, 28, 531-534.

8. Dickinson, A., Nicholas, D.J. & Adams, C.D. (1983) The effect of instrumental training contingency on susceptibility to reinforcer devaluation. Quarterly J Exp Psychol, 35, 35-51.

9. Everitt, B.J., Belin, D., Economidou, D., Pelloux, Y., Dalley, J.W. & Robbins, T.W. (2008) Review. Neural mechanisms underlying the vulnerability to developing compulsive drug-seeking habits and addiction. Philos Trans R Soc Lond B Biol Sci, 363, 3125-3135.

10. Horner, K.A., Logan, M.C., Fisher, T.J. & Logue, J.B. (2017) Blockade of patch-based mu opioid receptors in the striatum attenuates methamphetamine-induced conditioned place preference and reduces activation of the patch compartment. Eur J Pharmacol, 796, 207-214.

11. Murray, R.C., Logan, M.C. & Horner, K.A. (2015) Striatal patch compartment lesions reduce stereotypy following repeated cocaine administration. Brain Res, 1618, 286-298.

12. O'Dell, S.J. & Marshall, J.F. (2000) Repeated administration of methamphetamine damages cells in the somatosensory cortex: overlap with cytochrome oxidase-rich barrels. Synapse, 37, 32-37.

13. Yin, H.H. & Knowlton, B.J. (2006) The role of the basal ganglia in habit formation. Nat Rev Neurosci, 7, 464-476.

14. Zapata, A., Minnery, V.L. & Shippenberg, T.S. (2010) Shift from goal-directed to habitual cocaine seeking after prolonger experience in rats. J Neurosci, 30, 15457-15463.

15. Belin, D., Jonkman, S., Dickinson, A., Robbins, T.W. & Everitt, B.J. (2009) Parallel and interactive learning processes within the basal ganglia: relevance for the understanding of addiction. Behav Brain Res, 199, 89-102.

16. NIDA (2012). Principles of Drug Addiction Treatment: A Research-Based Guide (Third Edition). Retrieved May 9, 2017, from https://www.drugabuse.gov/publications/principles-drug-addiction-treatment-research-based-guide-third-edition

17. Gerfen, C.R. (1989) The neostriatal mosaic: striatal patch-matrix organization is related to cortical lamination. Science, 246, 385-388.