University of Colorado, Boulder
CU Scholar

Undergraduate Honors Theses | Honors Program

Spring 2011

A Novel Rodent Head-Fixed Go/No-go Contextual Discrimination Procedure to Identify a Role for the Subiculum in Reward-Related Working Memory

Samuel David Dolzani
University of Colorado Boulder

Follow this and additional works at: http://scholar.colorado.edu/honr_theses

Recommended Citation
Dolzani, Samuel David, "A Novel Rodent Head-Fixed Go/No-go Contextual Discrimination Procedure to Identify a Role for the Subiculum in Reward-Related Working Memory" (2011). *Undergraduate Honors Theses.* Paper 699.

This Thesis is brought to you for free and open access by Honors Program at CU Scholar. It has been accepted for inclusion in Undergraduate Honors Theses by an authorized administrator of CU Scholar. For more information, please contact cuscholaradmin@colorado.edu.

A Novel Rodent Head-Fixed Go/No-go Contextual Discrimination Procedure to Identify a Role for the Subiculum in Reward-Related Working Memory

By

Samuel David Dolzani

Department of Psychology/Neuroscience

Dr. Donald Cooper, Department of Psychology/Neuroscience

Dr. Robert Spencer, Department of Psychology/Neuroscience

Dr. Jennifer Knight, Department of Molecular, Cellular, and Developmental Biology

An undergraduate honors thesis submitted to the

Faculty of the University of Colorado

in partial fulfillment of the requirement

for the degree of Honors Baccalaureate

October 28, 2011

Abstract

A powerful new contextual discrimination task has recently been developed by the Cooper Laboratory in order to study the brain motivation/reward circuitry underlying reward-related behaviors. This particular go/no-go task was specifically developed with the intent to study the contributions of certain brain regions to working memory, motivation, and impulsivity. The subiculum presents itself as a pivotal brain region to study during this reward-related task because of its implication in the brain motivation/reward circuitry. For this reason, we chose to pharmacologically inactivate the dorsal and ventral subiculum using the reversible $GABA_A$ agonist, muscimol, during different stages of training in the novel go/no-go task to reveal the functional role of each region. Preliminary data that we have collected supports previous evidence for anatomically partitioned roles for the dorsal and ventral subiculum. The dorsal subiculum appears to play a role in impulsivity, whereas the role of the ventral subiculum in the go/no-go task remains unclear. Future goals for this experiment are to develop a more comprehensive understanding of the brain motivation/reward circuitry and gain insight into the maladaptive plasticity that may contribute to motivational and impulsivity disorders.

Acknowledgements

I would like to express my sincere gratitude to my thesis advisor and mentor, Dr. Donald Cooper, for sharing with me his vast knowledge of science and giving me the opportunity to perform neuroscience research in his laboratory. Dr. Cooper has been constantly supportive of my experiment and has provided the resources I have available to me to conduct research.

Words cannot express how thankful I am for Dr. Shinya Nakamura for guiding me along the way every day that I am in the laboratory. Dr. Nakamura has taught me everything that I know about working in a neuroscience laboratory and conducting behavioral neuroscience research. Without Dr. Nakamura this project would not have been possible. His comprehensive knowledge of neuroscience has enabled the success of this project.

I also would like to thank all of the other employees in the Cooper Laboratory for their constant help and support when I have needed it. In particular, I would like to thank one of the other undergraduate researchers in the lab, Matthew Pomrenze.

Finally, I would like to express my gratitude to Dr. Spencer and Dr. Knight for participating in this project as thesis committee members. I cannot express how thankful I am for their time and willingness in encouraging my academic success in this project.

Contents

Chapter One:

Introduction and Background Information

General Overview

The functional role of specific brain regions in short-term working memory and attention remains a mystery. The goal of this study is to identify important brain regions to elucidate their role in behaviors critical for working memory. After a brain region is identified and the functional role that it plays in a specific behavior is hypothesized, experiments can be designed to test the hypothesis. Of great utility to the modern neuroscientist are experimental paradigms developed to establish which brain regions are responsible for certain neurological functions. An example of this type of paradigm is the go/no-go task, which has been shown to have a high level of construct validity and face validity (Winstanley, 2011).

A go/no-go paradigm can be used in numerous different contexts, such as the novel technique recently developed in the Cooper Laboratory. This specific version of the go/no-go task involves the use of head-restrained, behaving rodents discriminating between two different frequencies of tones that correspond to the "go" and "no-go" constituents of the paradigm. This particular go/no-go task was developed specifically with the intent to study the motivation/reward circuitry underlying reward-related behaviors. Measurements of several components of impulsivity, working-memory, and motivation can be studied using the novel go/no-go paradigm. Of particular interest is the interaction between the brain motivation/reward circuitry and impulse control. It is known that high levels of impulsivity are a clinically significant symptom for a range of psychiatric disorders such as attention deficit hyperactivity disorder, bipolar disorder, personality disorders, pathological gambling, and substance abuse (Winstanley, 2011).

The subiculum is a brain region implicated in impulsivity and is worthy of study in an appropriate go/no-go task because of its anatomical location in the hippocampal formation, as well as the lack of congruent information in the current literature highlighting the role that it plays in impulsivity and motivated behaviors. Pharmacological inactivation has proven to be a powerful method for understanding the functional role of specific brain regions, and, therefore, I chose to develop an experimental paradigm with the ultimate goal of inactivating the subiculum during a novel go/no-go task developed by the Cooper Laboratory.

Before any pharmacological intervention can occur, rodents must be trained to perform the novel task to a specific criterion in order to establish an effect of inactivation. Inactivating the subiculum may reveal its functional significance in relation to reward-related behaviors, as well as provide a more comprehensive scientific understanding of the brain's motivation/reward circuitry, which may be beneficial in the development of therapies for numerous psychiatric disorders.

The Hippocampal Formation

The hippocampal formation is a compound structure located in the medial temporal lobe, consisting of the CA1 and CA3 region of the hippocampus, subiculum, entorhinal cortex, and dentate gyrus (Potvin et al., 2010). This anatomical location of the brain is involved in working memory, short to long-term memory consolidation, spatial navigation, and attention (O' Mara, 2006). A particularly interesting region of this brain structure is the hippocampus proper, which is crucial to working memory and has its primary outputs directed to the medial prefrontal cortex (mPFC), nucleus accumbens (NAc), and the subiculum. The mPFC has been studied extensively and has

been shown to play a key role in encoding affective information, which allows for the execution of adaptive behavioral responses (Hanes and Arnsten, 2009). The NAc has been identified as part of the limbic system, and plays an important role in reward-related behaviors, substance abuse, and addiction (Cooper, 2002). The hippocampus proper is critically involved in processing information that is subsequently encoded into long-term memories. The subiculum projects to the aforementioned cortical and subcortical structures within the limbic system, yet it remains unknown how the hippocampal information is processed and relayed through the subiculum (O' Mara, 2006).

A search of relevant literature uncovers numerous publications that illustrate "wiring diagrams" of the hippocampal formation completely omitting the central positioning of the subiculum in the hippocampal circuit. Hippocampal circuitry diagrams consist of CA3 projecting to the dentate gyrus projecting to CA1 with outputs to the entorhinal cortex (O' Mara et al., 2009). Ironically, the major output from CA1 is to the subiculum, while the output to the entorhinal cortex is only minor in comparison (O' Mara et al. 2009). The CA1 region of hippocampus sends its major outputs to the subiculum, which then projects to cortical and subcortical brain regions, making the subiculum the primary output structure of the hippocampus (O' Mara, 2001).

Anatomy and Physiology of the Subiculum

The subiculum is a pivotal, but under-investigated brain structure that plays an integral role mediating hippocampal-cortical and hippocampal-subcortical interaction (O' Mara, 2005). It has received nowhere near the level of scientific investigation as the surrounding regions of the hippocampal formation, and thus there is still very

much information missing that would allow scientists to generate a comprehensive theory about its role in the hippocampal formation and more generally speaking, its contribution to behavior (O' Mara, 2001). The homogeneity of the dorsal and ventral subiculum, that is, being a part of the same brain structure, should not lead one to assume that both regions contribute to the same aspects of behavior. The dorsal and ventral subiculum distribute their efferents quite differently and therefore contribute to different behavioral aspects (Andrzejewski et al., 2006).

An early study reported an association between subicular neuron firing and presentation of a tone paired with a food reward, which hinted that there is reward-related activity present in the subiculum (Segal, 1972; O' Mara, 2009). More recently, implications to the functional role of the subiculum have been presented in numerous studies with substantial evidence suggesting that the subiculum plays a key role in the brain motivation/reward circuitry (Cooper et al., 2006). It has been shown that neurons in the ventral subiculum increase their activity in response to reward anticipation, reward presentation, and spatial location (Cooper et al., 2003; Martin, 2001). Similarly, through the use of high-resolution single-cell recording techniques Cooper et al. correlated the ventral subiculum to novelty and drug seeking behavior, however, the role of the dorsal subiculum in reward-related behavior remains largely unknown (Cooper et al., 2006).

The ventral subiculum (vSUB) is an interface between the hippocampal formation and the NAc and the prefrontal cortex (PFC) (Cooper et al., 2003). This region has been shown to robustly activate the dopamine system, primarily in response to novelty, as well as providing a regulatory role in the inhibition of the HPA

axis. (Cooper et al., 2002; O' Mara, 2005). On the other hand, the dorsal subiculum (dSUB) processes information related to movement, memory, and spatial orientation (O' Mara 2005).

It has been hypothesized that the subiculum is the site of integration between hippocampal spatial information and whole-body movement information of cortical origin (Andrzejewski, 2006). Deadwyler and Hampson (2004) demonstrated that subicular and hippocampal neurons fired during a delayed nonmatch to sample (DNMS) task in response to the firing pattern during the previous trial. In this task rats were trained to attend to a stimulus that disappeared, then a delay was imposed before they could respond by lever pressing associated with a stimulus that did not match the initial stimulus. Thus, in the DNMS task short-term working memory is needed to span the delay period. For trials in a particular DNMS task that consisted of short (<15 s) delays, subicular cell firing in the proceeding trial approached a maximum firing rate, whereas hippocampal firing rates remained at a baseline level. However, prior trials with long (>15 s) delays resulted in decreased rates firing of subicular neurons and increased firing rates of hippocampal neurons (Deadwyler and Hampson, 2004). These results implicate partitioned roles a for hippocampal and subicular neurons in the encoding of memories related to the duration of working memory.

Some of the most compelling evidence in support of these differing roles comes from the inactivation of the subicular region using the $GABA_B$ receptor agonist, baclofen, which strongly reduced performance during short, but not long trials, in the DNMS task (Hampson and Deadwyler, 2003). Worth noting is the role of the subiculum in the behavioral inhibition system. Within this highly complex system, the

subiculum receives information about available goals from regions of the brain involved in planning of motor action. Prior to reaching the subiculum, unimportant and familiar information is filtered in the hippocampus, passing only important information on to the subiculum. The apparent job of the subiculum is to then compare and integrate this information and produce an output when conflict between incompatible goals is encountered (McNaughton, 2006). For instance, in a go/no-go behavioral task where the rat has to attend to a stimulus that either tells them to respond or withhold responding, there may be conflict between withholding from pressing a lever and receiving a reward or pressing the lever and not receiving the reward. Accordingly, this subicular output may allow the rat to inhibit its actions depending on the contingencies in place. Dysfunctional impulsivity, or inability to withhold responding during the no-go component of the task would result in failure to receive reinforcement for a behavior.

In order to understand the mechanisms underlying motivation and reward-related behavior, it is important to understand the content that specific regions of the reward circuitry transmit. By using a rodent model to investigate certain regions of the brain, it is possible to establish complex behaviors and collect information about multiple aspects of the particular behavior. Once the behavior has been established to a certain criteria, the brain region of interest can be manipulated electrically, physically, or pharmacologically.

The anatomical position of the subiculum makes total simultaneous inactivation of the dorsal and ventral subiculum extremely difficult. This would require multiple microinfusions and increases the possibility of interrupting surrounding brain regions.

For this reason, there have been very few ablations or inactivations of the entire subiculum (O' Mara, 2006). For the purposes of my experiment, I chose to bilaterally implant guide cannulae into the dorsal and ventral subiculum in different rats, rather than both regions in the same subject, because of my desire to elucidate the functional roles that each individual region plays in the specific reward-related task. In the current literature, there are no studies implementing the novel and powerful training procedure developed for this behavioral experiment in the Cooper Laboratory.

Chapter Two:

Methods

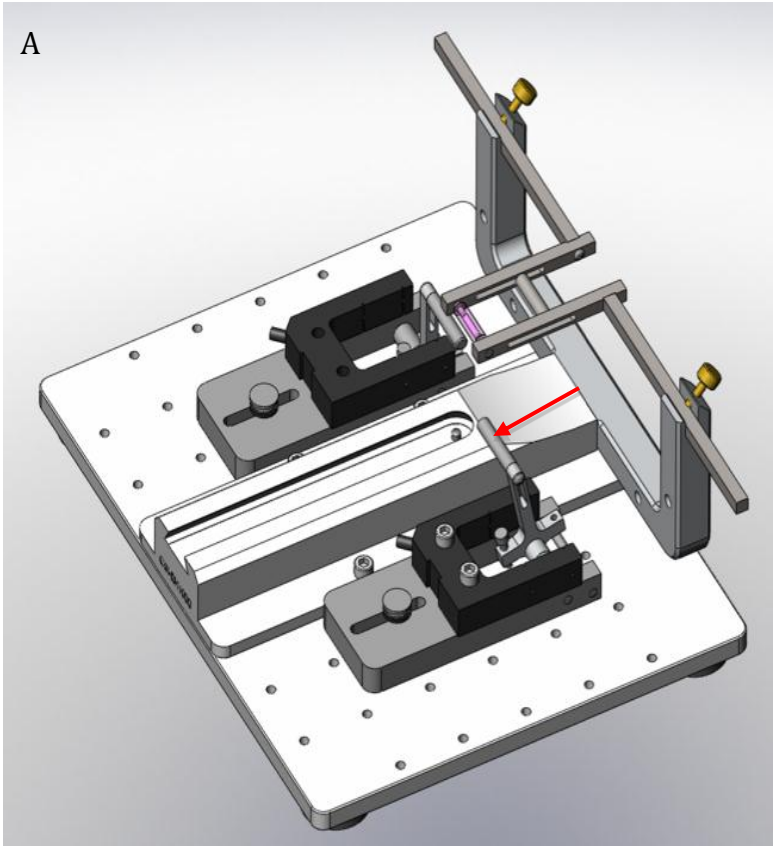
Subjects

Male Sprague Dawley rats (250-325 grams; Harlan, Indianapolis, IN) were used for the initial stages of the experiment. Male Long-Evans rats (250-325 grams; Harlan, Indianapolis, IN) were also used during the later stages of the experiment. Rats were singly housed on a twelve-hour reverse light/dark cycle (lights on at 8 p.m.). Food was available *ad libitum*, while water was only available after the last training session of each week from approximately 5 p.m. Friday until 12 p.m. Saturday. Restrictions on water intake were imposed in order to ensure that motivational levels were high during training days. All experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Colorado at Boulder.

Head-restraint Apparatus and Electronics

The training apparatus used throughout the duration of the experiment is a nickel-plated steel base-plate designed by Dr. Donald Cooper and manufactured by Old School Industries, Dacono, CO. The entire head-restraint device is comprised of a metal base-plate with a flexible plastic body-restraint wrap, and horizontal bars designed to attach to the head-restraint bars implanted on the rat's skull. The metal plate is designed with the intent to comfortably restrain the rat during the entire duration of the experiment. There are two forepaw-activated levers within close reach of the rat while he is restrained. For the purpose of this experiment, only the right lever was active and the left was fixed in a set position. The levers are coupled to infrared sensors that are activated when an infrared beam is disrupted. Directly in front of the rat's mouth is a sucrose delivery nozzle. The nozzle is wired to a solenoid

that regulates the amount of time in which the valve is open, and thus regulates the volume of sucrose administered. An infrared sensor connected to the reward-delivery system was used to count the number of times the rat licks the reward delivery nozzle (Figure 1). Shinya Nakamura, a postdoctoral fellow in the Cooper Laboratory, developed all of the software used for this experiment using LabView (National Instruments, Austin, Texas). The software computed the number of licks per training session, the number of lever presses, and served as the interface in which parameters of the go/no-go training task were established for each training session. Tones were presented to the rats using a small set of computer speakers connected to an Acer Eee desktop computer (Acer Incorporated, New Taipei City, Taiwan).



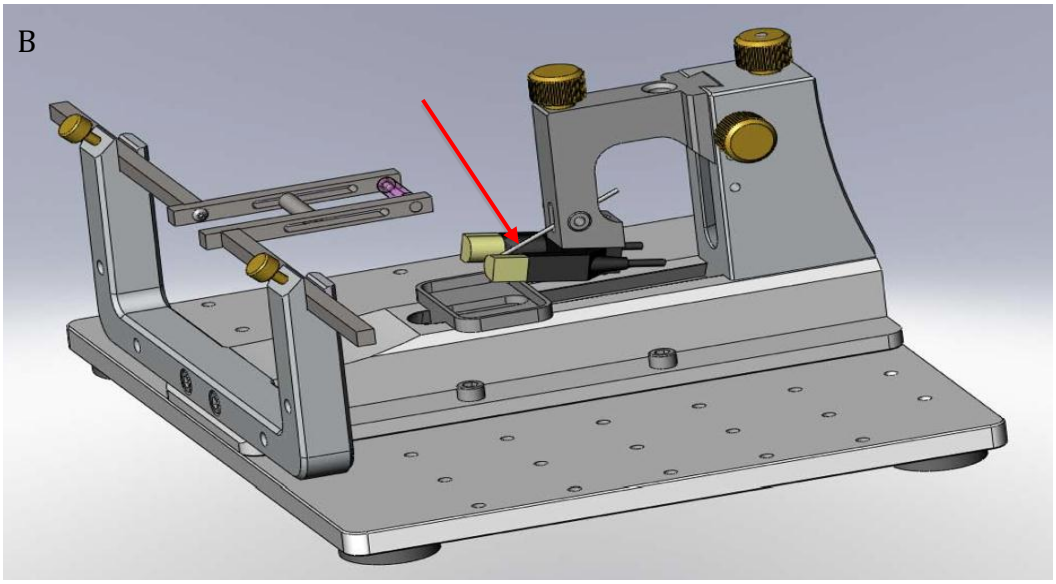


Figure 1. A. The head-restraint apparatus with forepaw levers. Red arrow is pointing to right forepaw lever. B. The head restraint apparatus with reward-delivery sensor and nozzle. Red arrow is pointing to the reward-delivery sensor/nozzle.

Surgical Preparation

Rats were placed into an acrylic chamber where they were administered the inhalation anesthetic, isoflurane, to initially anesthetize them. Once anesthetized, rats were administered an intraperitoneal injection of ketamine/xylazine. Ketamine is used for maintenance of general anesthesia and acts as an analgesic. Xylazine is used for analgesia, muscle relaxation, sedation, and anesthesia. Rats were administered the anesthetic cocktail based on their body weight at the time of surgery. Once anesthetized, hair was shaven off the entire ventral portion of the skull as well as around the ears, and the region was sterilized using 70% ethanol followed by iodine. Rats were then inserted into a stereotaxic device where their skull remained horizontal (bregma level with lambda) and immobilized for the entire length of the surgery. Below each rat during the surgery was a heating pad in order to maintain body temperature during the surgery. An incision was made in an anterior to

posterior fashion in the middle of the scalp approximately 2.5 cm long in the area of the scalp between the eyes, extending towards the most posterior part of the skull. Once the incision was made, tissues connecting the skull to the skin were removed. 0.9% saline was used to clean the area where tissues were removed. In order to remove any remaining tissue from the exposed region of the skull, 30% hydrogen peroxide was applied directly to the skull using cotton swabs. Hydrogen peroxide disinfects the region, eliminates remaining connective tissue, and cauterizes the vasculature on the surface of the skull. The use of hydrogen peroxide ensures that bleeding from the skull has ceased and allows for the skull to be desiccated before the next step of the surgery. After removing all connective tissue and eliminating any residual bleeding from the skull and surrounding tissues, the skull was once again cleaned with 0.9% saline and dried using cotton swabs (Figure 2).

In order to secure the stainless-steel head-restraint bars, seven screws must first be implanted into the skull. Four small screws were implanted between 5-10 mm anterior to the bregma in a small trapezoidal formation. Three more screws were implanted posterior to the lamba in a triangular formation, in regions of the skull where the bone is the thickest (Figure 2). Prior to screwing the screws into the skull, the bottom of the screws were covered with super-glue to increase their strength in adhering to the skull and increase the structural integrity of the head-fixation bars.

Once the screws were in place, the stereotaxic coordinates specific to the dorsal and ventral subiculum were marked on the skull. The coordinates used for implanting cannulae into the dorsal subiculum were –6.0 mm posterior to the bregma, 3.5 mm relative to the midline, and-2.0 mm ventral to the dura mater. The coordinates used

for the ventral subiculum were –6.7 mm posterior to the bregma, 5.0 mm relative to the midline, and -5.0 mm ventral to the dura mater. Small holes were drilled in the respective positions on the skull corresponding to the either the dorsal or ventral subiculum. Guide cannulae (Plastics One, Roanoke, VA) were implanted bilaterally into the subiculum through these holes (Figure 2). They were cemented into place using acryloplastic dental cement. Once bilateral guide cannulae were implanted, the stainless-steel head-restraint bars were lowered into place directly above the area where the screws were implanted, allowing the guide cannulae to be positioned between the two horizontal bars. The head-restraint bars were connected to the skull using acryloplastic dental cement. Cement was applied between the skull and the bottom of the head-restraint bars, allowing for the head-restraint bars to be positioned approximately 4-5 mm ventral to the surface of the skull. The approximate time for one surgery was 2-3 hours. Once surgeries were completed, rats were given 5-7 days recovery time with water and food available *ad libitum*.

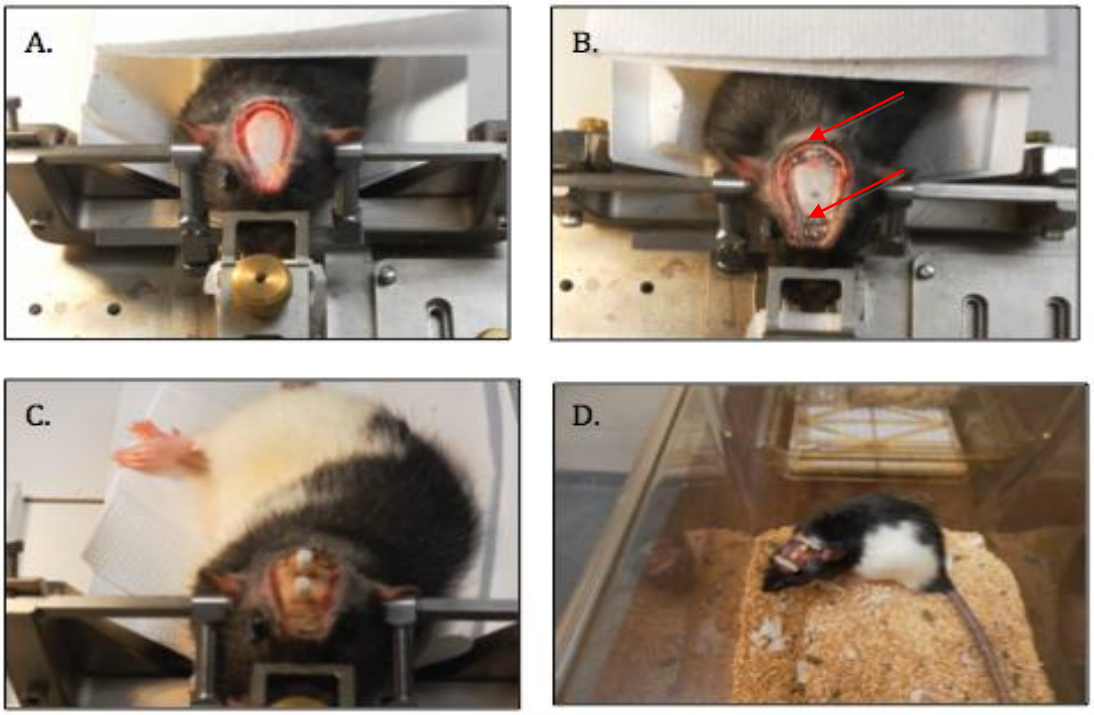


Figure 2. A. Tissues were removed from the skull and hydrogen peroxide was applied. B. Seven screws were implanted into the skull anterior to the bregma and posterior to the lamba (Arrows are pointing to the screws). C. Guide cannulae were implanted bilaterally into the brain region of interest and bonded to the skull using dental cement. D. Rodent with implanted head-holder bars and bilateral guide cannulae after 1 week of recovery time.

Lever Pressing Acquisition Stage of Training

Rats were handled and acclimated to the training boxes over the course of three days. Once placed in the box, rats were administered a 0.3 M sucrose solution through a syringe in order to prime them for the future reward delivery system. After three days of this condition, the rats were restrained for the first time. For 60 minutes each day, the restrained rats were administered sucrose non-contingently every fifteen seconds through the reward-delivery nozzle positioned near their mouth. Because of the initial discomfort in this immobilized state, the time needed for a rat to acquire the reward delivery nozzle licking behavior varied from subject to subject. When the rats performed this behavior for three days, the next step of the training began. The lever

pressing condition was introduced after successful reward consumption. We chose to use the right forepaw lever so the left side of the device could be used as a platform for the rat to support their body while pressing the lever. To initiate lever pressing, the lever was pressed for the rat five times at the start of each training session until the rat learned the association between lever pressing and reward-delivery. During this period of training, the inter-trial interval (ITI) was set at 1 s, so the rat was able to obtain the sucrose reward one time per second upon correct lever pressing. This training condition was maintained for as many days as necessary until the rat pressed the lever >1000 times for 2-3 consecutive days.

Lever + Tone Training Condition

After successful lever pressing for 2-3 days, rats were introduced to the two tones that were used for the remaining period of the training. The "go" and "no-go" tones were 9000 Hz and 3000 Hz, respectively. The two tones are readily discernible and are in the audible spectrum of sound frequencies for humans and rats, which make them good choices for this particular task. During this period of training, rats are restrained in the device for 60 minutes per day. Either the go or no-go tone are presented to the rat for 4 seconds and during this period the rat must press the right forepaw lever one time to receive a reinforcement of sucrose solution. The rat is no longer able to receive reinforcement non-contingently for his behavior; sucrose reward delivery is now contingent on lever pressing during either tone. This period of the training is designed to instill within the rat the association between lever pressing and reward delivery. Each individual rat is trained using the aforementioned parameters until they respond correctly to the tone at least 75% of the trials per

training session for at least two consecutive training sessions. Once this criterion is reached, the no-go portion of the training begins.

"No-go" Training

This step of training was chosen to follow immediately after the "lever + tone" portion of training because it is presumably the more difficult behavior to acquire of the two constituents of the final go/no-go task. This phase involves behavioral inhibition in which the subject must withhold from pressing the lever during a period of white noise, whereas the go portion of the training does not. A 3000 Hz initiation tone is presented for 2 seconds and the rat must press the lever during this period to receive a small reward that is thought to prime them for the final reward they receive when they successfully complete the no-go trial. There is a 1 second delay after the initiation tone, followed by a 4 second period of white noise. During the period of white noise, the subject must withhold from pressing the lever. If he presses the lever during this period, the white noise will terminate and there will be a 20 second ITI. Upon refraining from pressing the lever during the period of white noise, there is a one second delay followed by the final 3000 Hz tone. The subject must press the lever during this tone to receive the final reward, which is 5 x the sucrose volume of the initial reinforcement. After responding correctly, there is a 15 second ITI followed by the next trial's initiation tone. To summarize, correct responding during no-go trials requires 2 lever presses and abstaining from lever pressing during the period of white noise. Once the subject is able to perform the no-go portion of the training using the 4-second period of white noise at least 70% of trials per training session for at least two consecutive days, the white noise period is reduced to 2 seconds and remains this

duration for the rest of the training process (Figure 3 B.). The go portion of training begins after the subject has performed this criterion correctly at least 70% of the trials each training session for at least 2 consecutive days.

"Go" Training

During this phase of training subjects are presented with the 9000 Hz go tone. The rat must press the lever during the 2-second initiation tone in order to receive the first reinforcement of sucrose solution. If the subject responds correctly during the first tone, there will be a 1 second delay and then white noise will be presented indefinitely until the lever is pressed. This method of using indefinite white noise was implemented with the intent to encourage lever pressing during the period of white noise. The go component of the training requires the rat to press the lever during this period of white noise in order for the final reward tone to be presented. After successfully pressing the lever during the period of white noise, the final tone is presented and the rat must press the lever during this tone to receive the final reward. If he fails to press the lever during the first tone there is a 20 second ITI before the start of the next go trial. The indefinite period of white noise is only temporary and is not used in later stages of training. This phase of training is maintained until the rat responds correctly during the period of white noise over 70% of trials per training session for at least two consecutive days. Once this criterion is met and behavior is stable, the indefinite period of white noise is reduced to 2 seconds (Figure 3 A.). Failure to press the lever during this 2-second period of white noise results in a 20 second ITI. In summary, the rat must press the lever during the initiation tone, the period of white noise, and the final reward tone to successfully complete a go trial.

After successfully completing the go portion of training at least 70% of all trials per training session for at least 2 consecutive days using the 2-second period of white noise, the consecutive stage of training begins.

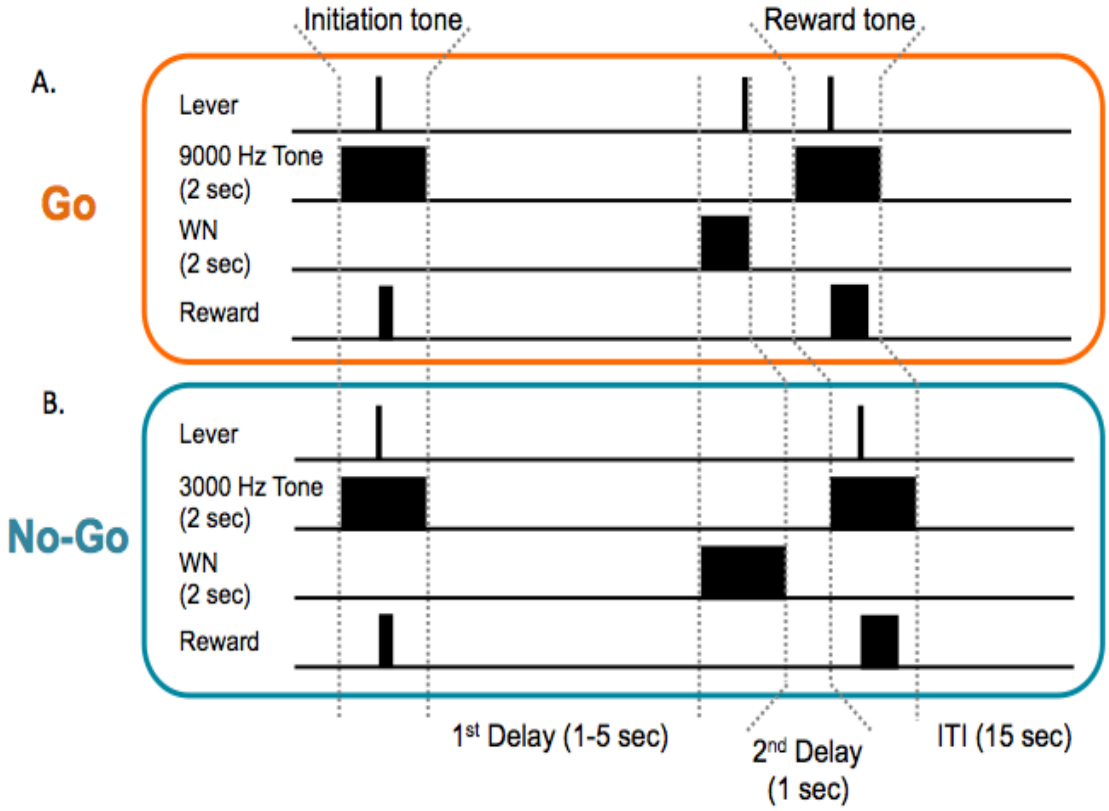


Figure 3. The rat is required to press the lever during the initiation tone to start the trial. During the period of white noise (WN), pressing the lever (go) or withholding it (no-go) is required. Reward tone is delivered if the rat responds correctly during WN.

Consecutive Go/no-go Training

This phase of training proceeds the go-only phase of the training procedure only after go-only behavioral performance is maintained at >70% correct responding for at least 2-3 consecutive training sessions. During this period the subject is initially presented with either a go or no-go trial. If a go trial is presented initially, the subject must respond and if the subject responds correctly the next trial will also be a go trial. The subject will be presented with go trials until they respond correctly to three

consecutive go trials. Once this is achieved, no-go trials will be presented and the subject must respond to three consecutive no-go trials in order to progress to the next go trial. For the initial stage of consecutive go/no-go training there is an indefinite period of white noise during "go" trials. Only when the rat responds to three consecutive go trials within 2 seconds of the initiation of the white noise will they move to no-go trials. The purpose of the consecutive go/no-go training is to facilitate acquisition of discrimination between the two different tones when they are presented in the same training session. This phase of training poses a challenge for subjects because it is the first stage of training in which they must discern between two different tone frequencies and initiate an appropriate response. Each session during this phase of training lasts for 90 minutes. Once subjects have performed this phase of the training correctly 80-85% of the trials per training session for 2-3 consecutive days they begin training on the final stage of training: the random go/no-go sequence.

Random Sequence Go/No-go Training

The random go/no-go training stage is the final stage of training in which the rat is presented at random with either go or no-go trials and must initiate the correct response in order to receive the sucrose reward. The computer program used randomly selects the trial condition, but it is set to maintain an equal balance of go and no-go trials throughout the course of each 90-minute training session. During the initial stages of this phase of training, a random 1-3 s delay following the initiation tone was used. This was used to minimize the likelihood of the subject chaining together specific behaviors in order to "cheat" their way through the task. Our concern was that the subject could potentially use a system of licking the reward-delivery nozzle in an

instrumental fashion without actually using working memory to determine and initiate the appropriate response during the period of white noise. Therefore, a random delay period would inhibit this behavior from working to the subject's benefit. After successfully responding to 80-85% of trials per training session using the 1-3 second random delay, the random delay period interval was increased to 1-5 seconds. The goal of expanding the random delay period was to train subjects in a way that would utilize working memory, while reducing instrumental behavior. Combining the go and no-go components into a random sequence is ideal for pharmacologically inactivating the subiculum prior to the behavioral task. The random sequence allows for the researcher to measure changes in impulsivity and motivation within the same subject between training sessions. The entire training protocol takes approximately 3 months of 5 days/week daily training sessions to complete.

Muscimol Microinfusions into the Subiculum

The $GABA_A$ agonist, muscimol (1 μg/μl), was injected bilaterally into the dorsal and ventral subiculum. Muscimol is an ideal substance to reversibly inactivate the subiculum because it is only active in the subject's brain for approximately 24 hours and, therefore, the brain region can be inactivated during numerous different training sessions and training conditions. This enables one to study the behavioral effects of muscimol infusion during one training session and then analyze the behavior during the following training session when the muscimol is no longer exerting its effects. Muscimol was injected through bilateral guide cannulae into either the dorsal or ventral subiculum. As previously mentioned, cannulae were implanted using stereotaxic coordinates previously determined to be ideal for targeting the dorsal and

ventral subiculum. Subjects were wrapped in a cotton cloth for the entire duration of the injection procedure in order to reduce movement and potential for injection failure. Microinjectors were inserted into guide cannulae and 1 µl of muscimol (1 µg/µl) was infused over the course of 2 minutes into each individual side of the subiculum using a Nanoliter microinjector (WPI Inc., Sarasota, FL). Injectors remained in guide cannulae for 5 minutes, following each infusion, before removal in order to allow muscimol to diffuse throughout the target region. Subjects were returned to their home-cage for 10 minutes prior to the start of the behavioral task. This 10-minute period was intended to allow muscimol to exert its effects, as well as to becalm the subject after the experiencing the potential discomfort associated with the injection procedure.

Chapter Three:

Results

Subjects underwent a rigorous training procedure in order to prepare them for muscimol (1 μg/μl) inactivation of the dorsal and ventral subiculum. Data from representative subjects has demonstrated that the current experimental design possesses a high level of utility in facilitating the acquisition of a complex and sophisticated tone discrimination task involving behavioral inhibition and execution. The initial stages of training consisted of acquisition of lever pressing during the presentation of a tone. This stage of training proceeded until behavioral performance reached at least 75% correct responding for several days until performance became stable (Figure 4).

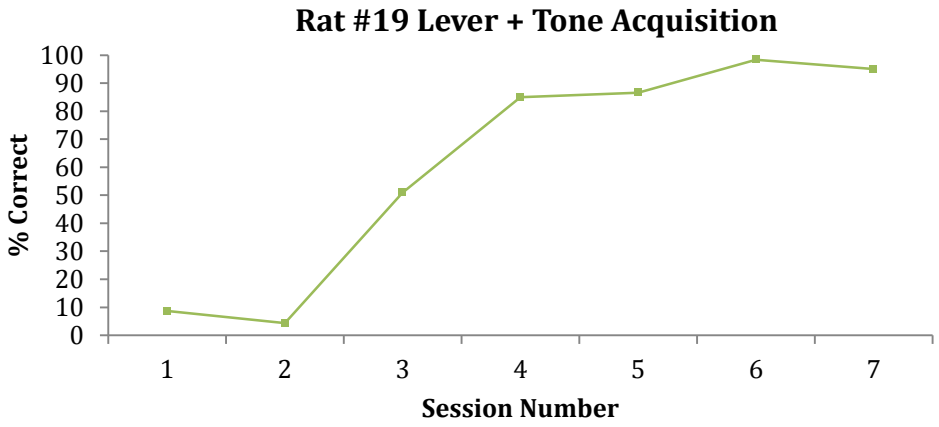


Figure 4. The lever + tone training condition for rat #19 proceeded for one week until the behavior was established at >75% correct responding. This is data from a representative subject.

Following the lever + tone acquisition stage of training, the rat was introduced to the no-go portion of the training process. This condition was maintained until the subject was able to respond to at least 70% of the trials correctly for two consecutive days and behavior was stable (Figure 5). The next stage of training was acquisition of the go constituent of the task. This stage of training was maintained until the subject

responded correctly to at least 70% of the trials per training session and behavior appeared to be stable (Figure 5).

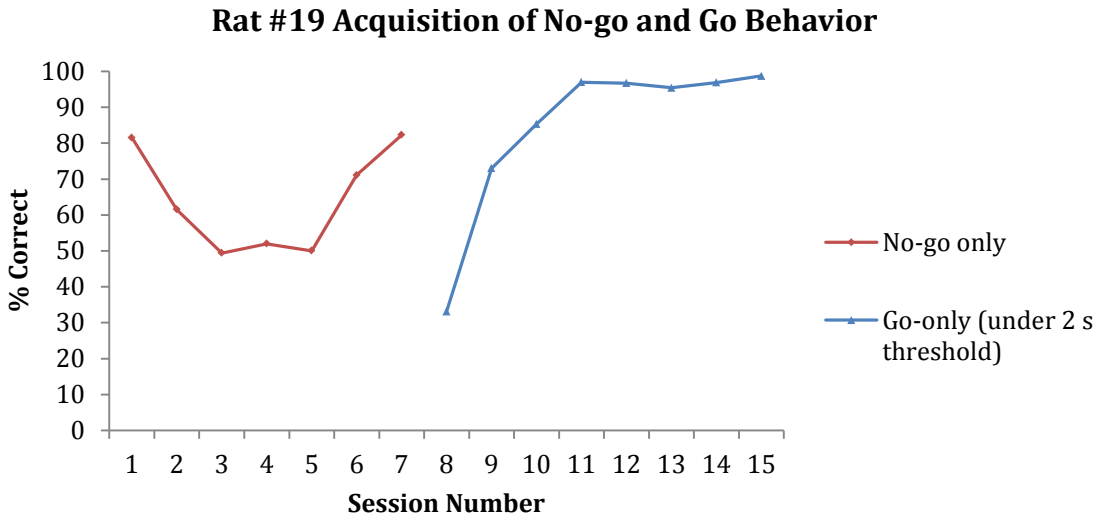


Figure 5. This representative data illustrates the subject's ability to acquire the "no-go" and "go" constituents of the behavioral task.

Once each subject was able to perform each constituent of the go/no-go task correctly, the consecutive go/no-go sequence was implemented. This stage of training proceeded until the subject was able to respond to both go and no-go trials correctly at least 70% of the trials per training session or when overall performance was 80% correct (Figure 6). The delay period between the initiation tone and the white noise remained at 1 s for the first portion of this training condition. After this behavior was established, the random go/no-go sequence of training began. Once overall performance of at least 80% correct during this stage of training was established, the delay period was switched from 1 s to a random 1-3 s then a random 1-5 s delay (Figure 6). As previously mentioned, the purpose of the random delay was to

decrease any linking of behaviors that might allow the subject to correctly respond without fully utilizing working memory.

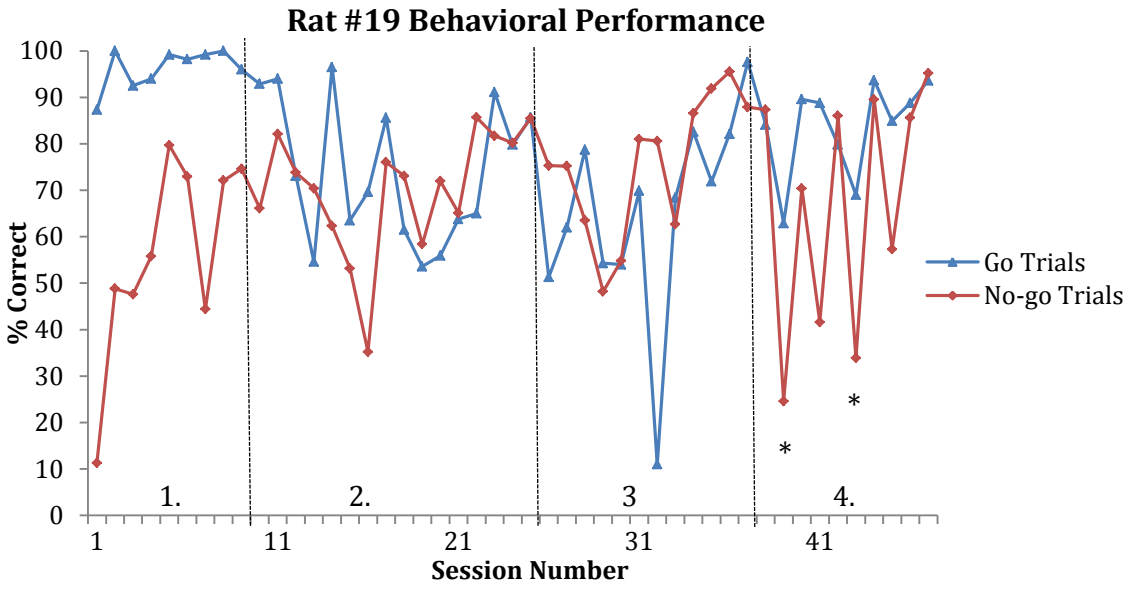


Figure 6. 1. This portion of the training procedure involved the go/no-go consecutive sequence. 2. During this stage of training, the subject experienced the random go/no-go sequence using a 1 s delay period. 3. After successful completion of random go/no-go training with a 1 s delay, the delay duration was changed to a random 1-3 s then 1-5 s delay. 4. This was the phase of the experiment in which control, muscimol, and recovery training sessions occurred. The delay period during this phase of the experiment was a random 1-5 s interval. Training sessions where muscimol (1 µg/µl) was infused are denoted with a *.

Due to the amount of time that it takes to train each subject to perform the go/no-go task to the criteria that we established, only three rodents have experienced muscimol inactivation of either the dorsal or ventral subiculum. Our initial goal was to train rodents until they were correctly responding at least 80% of the trials per session before introducing a manipulation using muscimol. Rat #19 was the first to meet this criterion, and was therefore the first subject to receive bilateral muscimol (1 µg/µl) infusion into the dorsal subiculum. As is shown in Figure 7 A, the percent of correct responses during the period of white noise during the two days of muscimol inactivation was drastically lower compared to control and recovery training sessions.

Of particular interest is the decrease in correct performance during the period of white noise in the no-go trials during the training sessions when the dorsal subiculum was inactivated (Figure 7 A). This decrease in behavioral performance was reversed the following recovery day, as the muscimol was no longer exerting its effects after 24 hours.

Because of the significant change in behavioral performance in rat #19, we decided to modify the task in order to increase the frequency of muscimol infusions throughout the training process. For rat #21 and rat #23, we chose to inactivate the subiculum during the initial stages of training. Thus far, we have bilaterally inactivated the ventral subiculum in rat #21 and the dorsal subiculum in rat #23 during the no-go phase of training. Data for these subjects is shown in Figure 7 B and 7 C. To determine what specific aspect of the behavior was affected as the result of muscimol infusion, we decided to analyze the performance during the first delay period. We believed that if impulse control decreased as a result of the inactivation of subiculum, the subject would press the lever more frequently during this time period. Inactivation of the dorsal, but not ventral, subiculum resulted in a significant increase in the percent of trials in which the lever was pressed during the delay period between the initiation tone and the period of white noise. Although the sample size for subjects with dorsal subiculum cannulae was only 2 and the sample size for subjects with ventral subiculum cannulae was only 1, the fact that the behavioral changes were reversed in each proceeding recovery day suggests that inactivating the dorsal subiculum decreased the subject's ability to withhold from lever pressing. This preliminary data supports the need for a larger sample size and we are currently training several other

subjects. Figure 8 shows the effect that inactivation of the subiculum had on lever pressing during the first delay period.

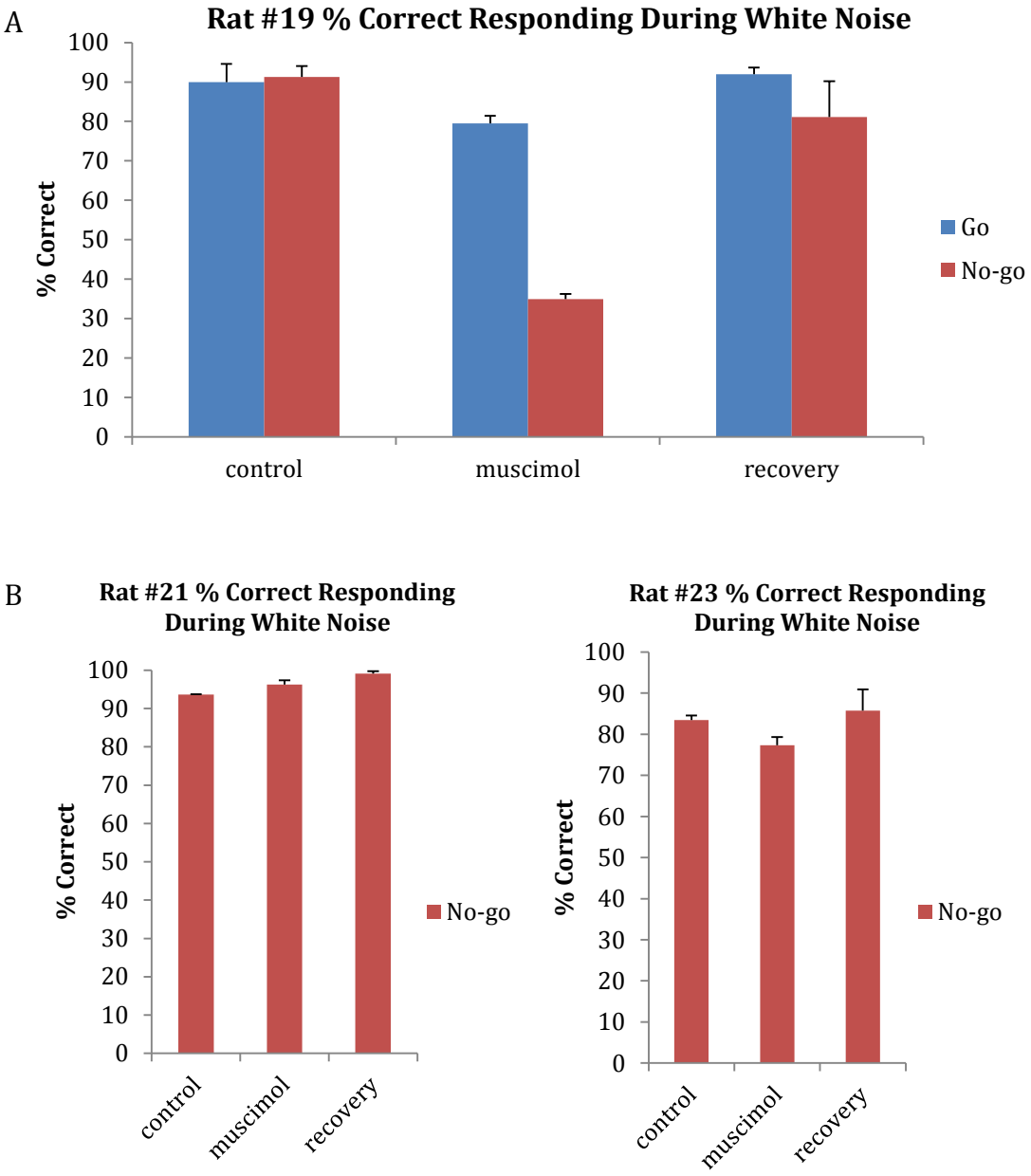


Figure 7. Behavioral performance is shown above for the three subjects who have undergone muscimol (1 µg/µl) inactivation of the subiculum thus far in the experiment. Rat #19 and rat #23 experienced bilateral inactivation of the dorsal subiculum, whereas rat #21 experienced bilateral inactivation of the ventral subiculum. Inactivation of the dorsal subiculum significantly affected behavioral performance during the period of white noise for Rat #19and Rat #23. Inactivation of the ventral subiculum did not result in a noticeable effect on behavioral performance during the period of white noise in rat #23. A. Rat #19 experienced 6 control training sessions, 2 muscimol training sessions, and 2 recovery training sessions. B. Rat #21 experienced 4 control training sessions, 2 muscimol training sessions, and 2 recovery training sessions. Rat #23 experienced 4 control training sessions, 2 muscimol training sessions, and 2 recovery training sessions.

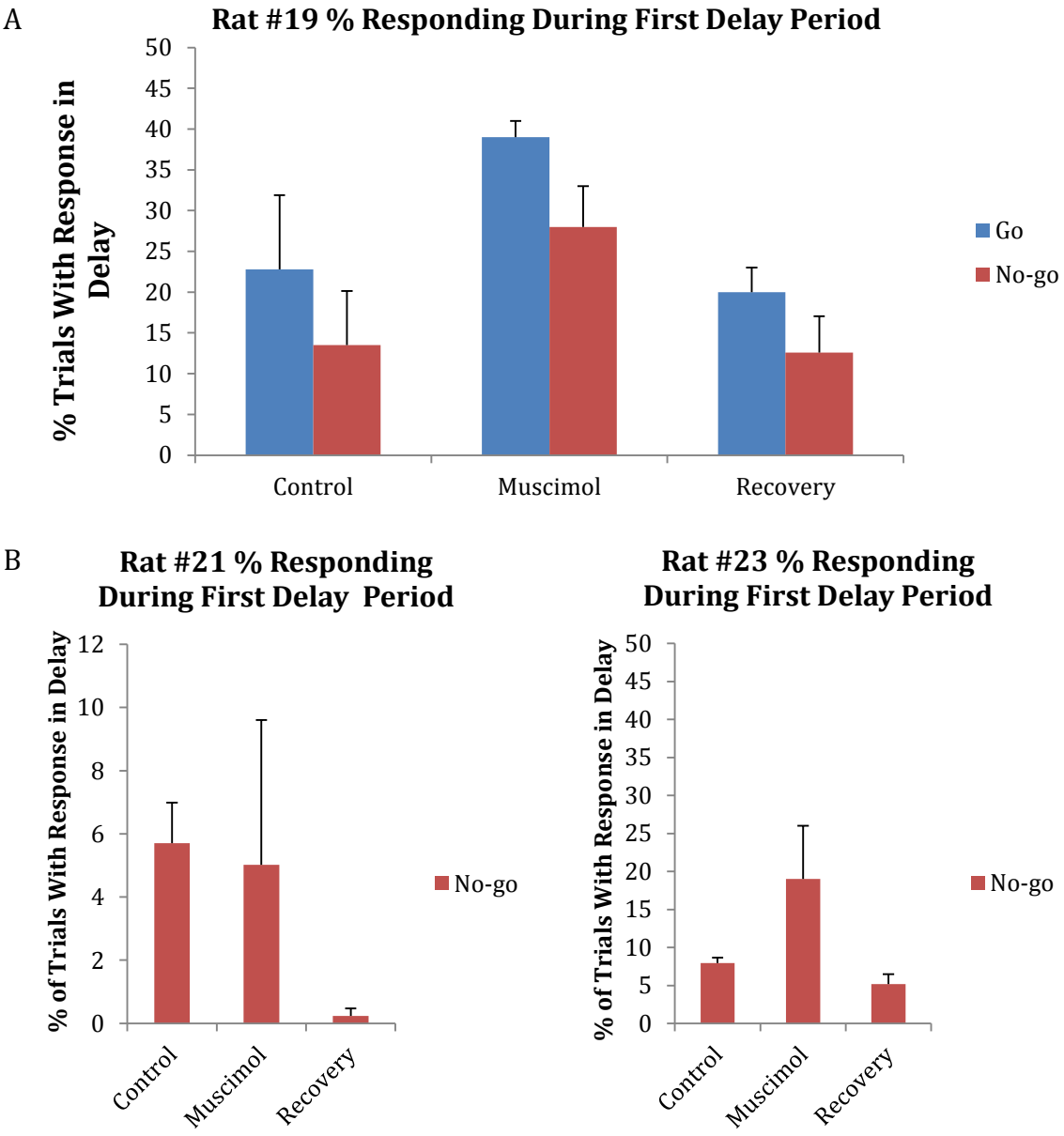


Figure 8. The data shown quantifies lever pressing during the first delay period for subjects with dorsal and ventral subiculum inactivation during the go/no-go task. Increased lever pressing can be seen in rat #19 and rat #23 during the training sessions in which muscimol (1 µg/µl) was infused into the dorsal subiculum. This preliminary data suggests a role for the dorsal subiculum in inhibitory behavior. Rat #21 received muscimol infusion into the ventral subiculum. A. Rat #19 experienced 6 control training sessions, 2 muscimol training sessions, and 2 recovery training sessions. B. Rat #21 experienced 4 control training sessions, 2 muscimol training sessions, and 2 recovery training sessions. Rat #23 experienced 4 control training sessions, 2 muscimol training sessions, and 2 recovery training sessions.

The purpose of using a random delay duration between the initiation tone and white noise was to reduce the likelihood that subjects were not performing the discrimination task using working memory, as well as to determine the effect that different delays had on the task performance. The random delay used for rat #19 was 1-5 s and the random delay used for rat #21 and #23 was 1-3 s. It is evident with rat #19 that behavioral performance decreased in trials with a delay of 5 s when compared to trials with a 1 s delay, particularly in no-go trials (Figure 9 A). This same relationship between delay duration and correct responses can be seen with rat #23 (Figure 9 B). This relationship was not as evident in rat #21 as in #19 and #23 (Figure 9 B). This may be due to the relatively small number of data points for each particular condition of training. This also may be due to the infusion of muscimol into the ventral subiculum in subject #21. Future research will consist of a larger sample size and more days of training in the control, muscimol, and recovery conditions.

The most compelling data from the three subjects who experienced muscimol infusion into the subiculum is that of subject #19. Subject #19 was trained using the random go/no-go sequence, meaning that he was presented with a situation where he had to respond correctly to either go or no-go trials in order to receive sucrose reinforcement. To visually represent his behavior during the control, muscimol injection into the dorsal subiculum, and recovery condition of the random go/no-go task, raster pots were created for 11 representative trials during each training condition for three consecutive days. The raster plots show the performance during each of the 11 trials (Figure 10 A-C). Lever pressing, licking, reward-delivery, white noise presentation, and tone presentation are all shown. During the control and

recovery training sessions, behavioral performance for both go and no-go trials was high, however, during the trials where muscimol was infused into the dorsal subiculum, go and no-go performance were both noticeably lower. Lever pressing is markedly increased during the trials in the training sessions where muscimol was infused into the dorsal subiculum. Behavioral data for each condition during these 11 representative trials is shown in Figure 10 D. Histograms are shown for all go and no-go trials during 1 control training session, 1 muscimol training session, and 1 recovery training session, respectively (Figure 11 A-C). The left histogram in each panel shows lever pressing data for all go trials in each training session and the right histogram in each panel shows lever pressing during all no-go trials in each training session. The number of lever presses during the white noise period of no-go trials in the muscimol training session (Figure 11 B) was substantially greater than the number of lever presses in the control and recovery training session during the white noise period of no-go trials (Figure 11 A and C). This is consistent with data in Figure 8 A in which lever pressing during the first delay period of no-go trials in muscimol training sessions was substantially higher than control and recovery sessions. Increased lever pressing during the period of white noise in no-go trials resulted in the subject not receiving the final sucrose reward and is considered a failed trial.

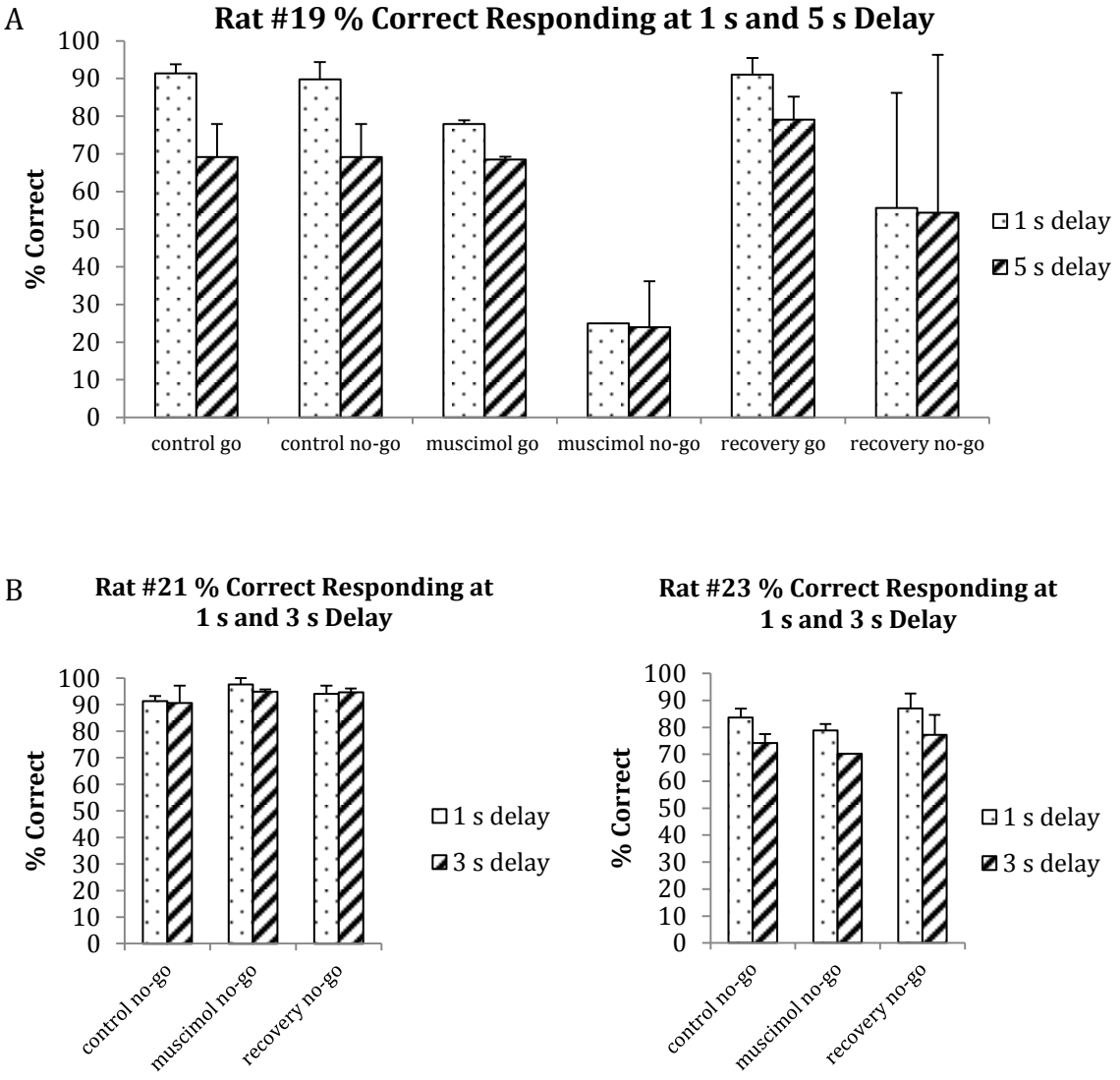
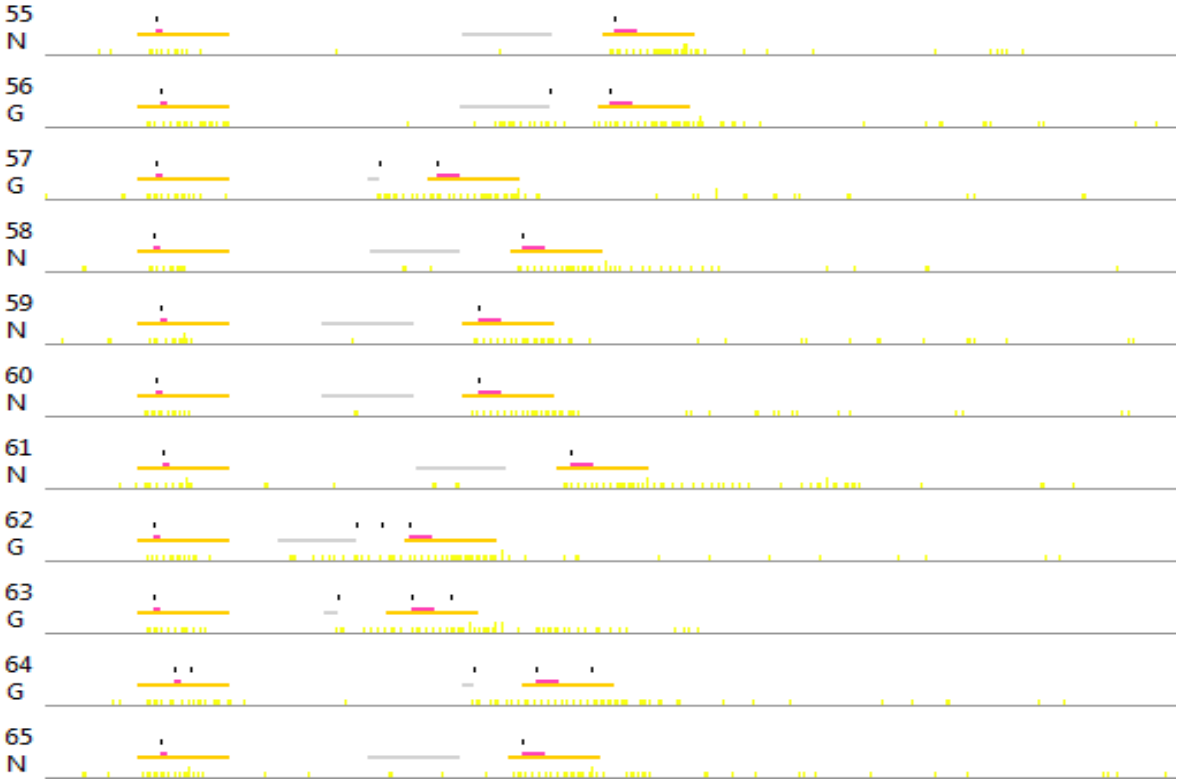
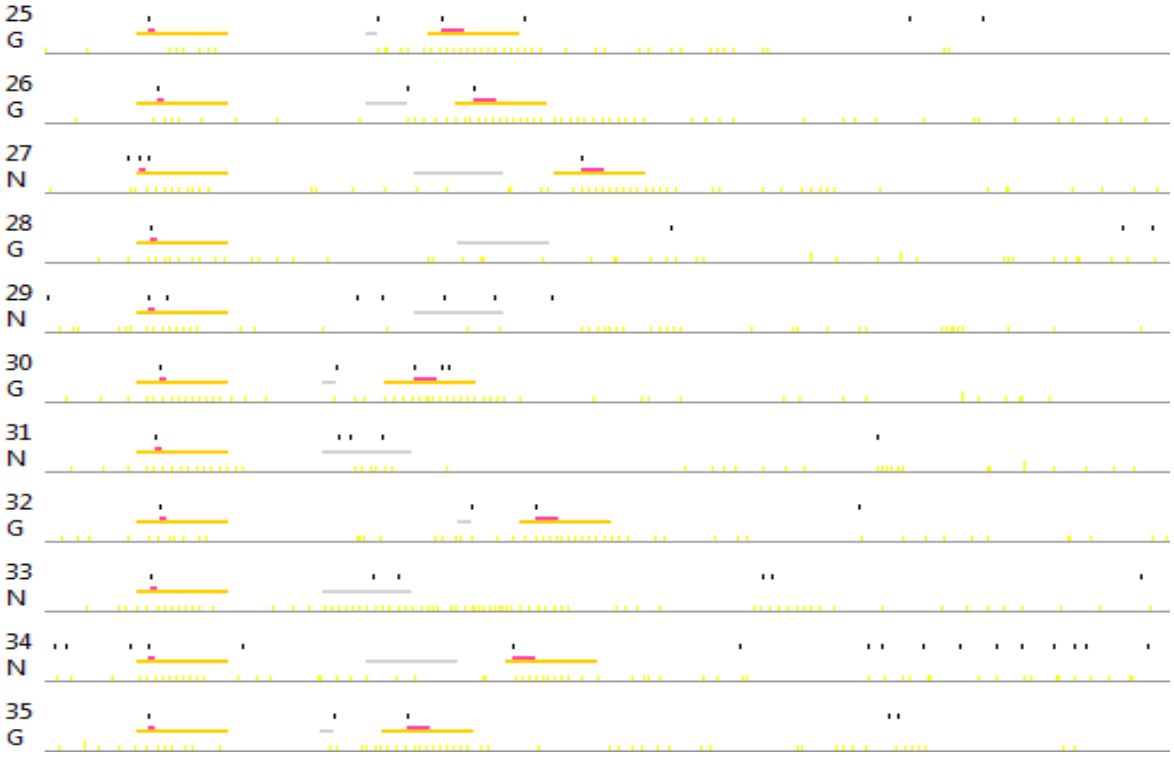


Figure 9. Behavioral performance is shown for the shortest and longest duration delay period that each subject experienced during control, muscimol (1 µg/µl), and recovery training sessions. For rat #19 and #23 behavioral performance was higher during the shortest delay period than the longest delay period. Behavioral performance for rat #21 was not directly related to the length of the delay period. These inconsistencies in the data may be due to the low number of training sessions in each particular condition, or may be the result of the inactivation of different regions of the subiculum. A. Shown is the mean percent correct responding for go and no-go trials during trials with trials a 1 s or 5 s delay period. Data for 6 control training sessions, 2 muscimol training sessions, and 2 recovery training sessions is included. B. Shown is the mean percent correct responding during trials with a 1 s or 3 s delay period. For rat #21 data for 4 control training sessions, 2 muscimol training sessions, and 2 recovery training sessions is included. For rat #23 data for 4 control training sessions, 2 muscimol training sessions, and 2 recovery training sessions is included.

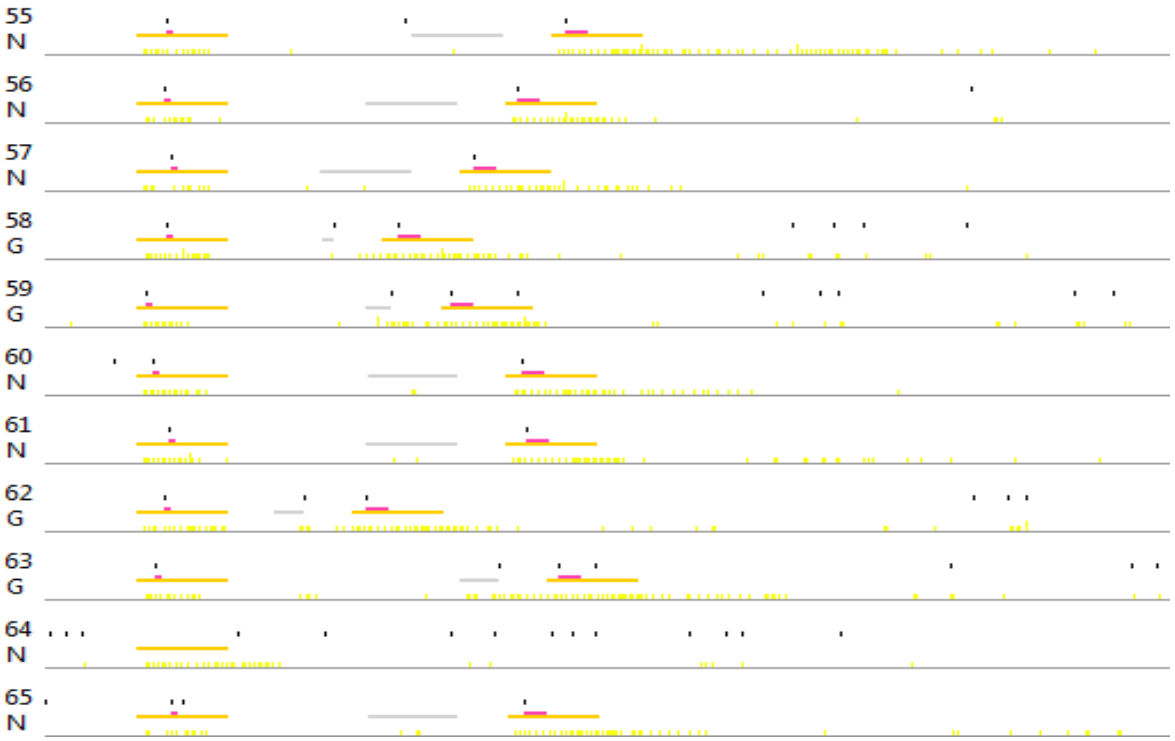
A


55
N
56
G
57
G
58
N
59
N
60
N
61
N
62
G
63
G
64
G
65
N

B


25
G
26
G
27
N
28
G
29
N
30
G
31
N
32
G
33
N
34
N
35
G

C



D

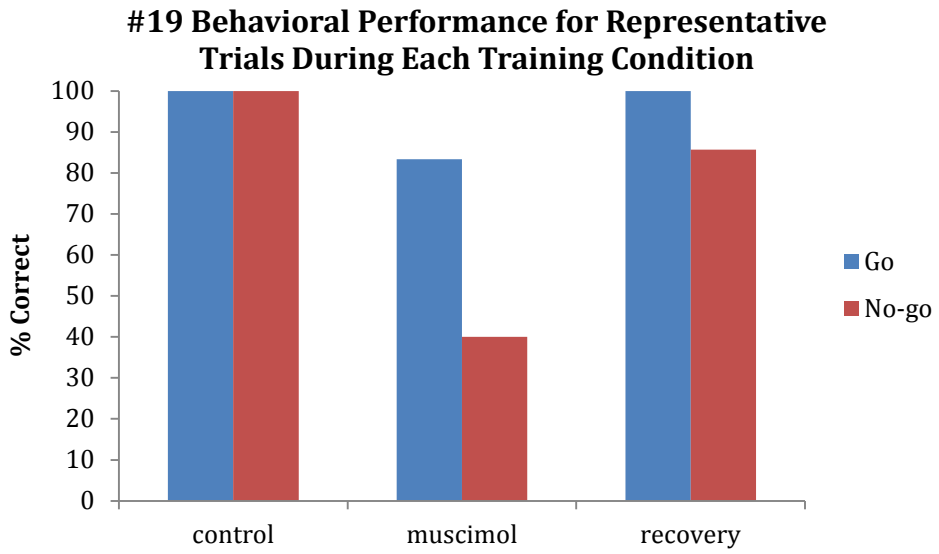


Figure 10. A-C: The trial number is listed on the far left. Below the trial number is a G or N, which denotes a go trial (G) or a no-go trial (N). The initiation tone is the first orange horizontal bar. The duration of this tone is 2 s. The delay period is the interval between the first orange horizontal bar and the subsequent grey horizontal bar. The grey bar represents the period of white noise and is only present if the subject correctly responded during the initiation tone. Black vertical marks represent each time the lever was pressed. Pink horizontal bars represent reward delivery. Yellow vertical marks represent licking. The ITI is represented after the orange horizontal tone presentation bars. A. This raster plot shows representative data for a control training session for subject #19. B. This raster plot shows representative data for a training session when muscimol (1 µg/µl) was bilaterally infused into the dorsal subiculum. C. This raster plot shows representative data from the recovery training session immediately following the muscimol condition. D. Behavioral performance is shown for each sequence of 11 consecutive trials for the three conditions shown in the A-C.

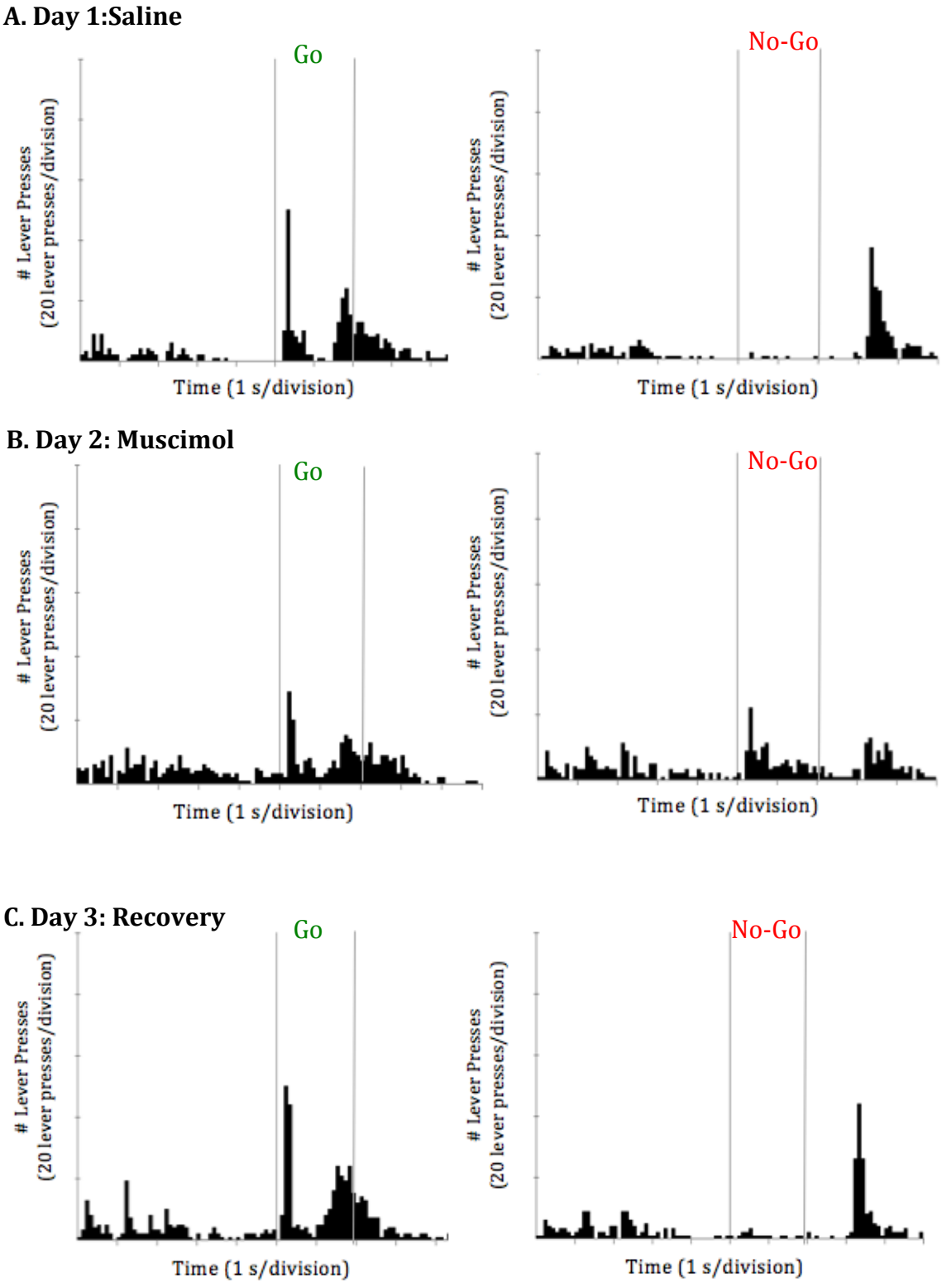


Figure 11. Shown is a histogram of lever pressing for 1 control training session (A), 1 muscimol (1 µg/µl) training session (B), and 1 recovery training session (C). The histogram in the left column of each panel shows lever pressing during all of the go trials per training session and the histogram in the right column shows lever pressing during all of the no-go trials per training session. The vertical grey lines represent the 2 s WN period in which the subject must press the lever during go trials or withhold from pressing the lever during no-go trials. (Bin size = 100 ms)

Chapter Four:

Discussion

Developing a complex, cultivated behavioral training paradigm is a difficult, yet requisite step in the process of studying reward-related behavior and its underlying neural correlates. The data collected in the preliminary stages of this ongoing experiment has substantiated the effective potential of our recently developed training procedure as reward-related behavioral task. Naïve rodents have been subject to operant conditioning in a situation where the contingency for reinforcement depends on their ability to properly discriminate between different tones and execute the response appropriate for the presented tone. The theory behind this type of experimental paradigm is that rats do not learn responses, they learn goals and therefore if they are motivated to achieve the end goal, such as a sucrose reward, they will behave in a manner that will maximize their chances of achieving the goal (McNaughton, 2006). With this theory in mind, we developed a rather complex behavioral model to test the motivational drive of the subject while exploiting several important aspects of behavior we wished to study.

The training model was designed with the intent to integrate working memory, motivation, and impulsivity in a way that could provide insightful information about the neural underpinnings of these components. Once trained to criterion, pharmacological inactivation of the subiculum became a useful tool for analyzing the functional role of the subiculum within these constituents of reward-related behavior. This gave us the ability to determine what, if any, role the subiculum contributed to certain aspects of the reward-related working memory task.

The preliminary results are encouraging, but not entirely conclusive due to the small sample size and lack of extensively repeated trials within each training condition.

Results from our training procedure suggest that the protocol being used to train rodents is effective in establishing a reward-driven behavior. The most compelling data comes from the subjects who experienced inactivation of the dorsal subiculum when compared with inactivation of the ventral subiculum. As previously stated, there appears to be anatomically generated segregation of function within the subiculum, that is, the ventral subiculum playing a role in the mediation of the hippocampal formation inhibitory control over the HPA axis, and the dorsal subiculum playing a role in the processing of spatial, mnemonic, and movement information (McNaughton, 2006).

The data collected showing that bilateral ventral subiculum inactivation had no noticeable effect on the components of behavior we were analyzing suggests that the task may not have robustly activated the ventral subiculum, or the activity occurring in the ventral subiculum during the task was not directly related to behavioral performance. On the other hand, inactivation of the dorsal subiculum resulted in an increase in behavior that exhibits properties dysfunctional impulsivity, which has been defined as fast and inaccurate responding where this is non-optimal (Kumari et al., 2009). The subjects who experienced inactivation of the dorsal subiculum showed a significant increase in lever pressing during the first delay, which models an impulsive decision that may affect the subject's ability to reach their goal. In go trials this does not pose a problem for the subject because there is no punishment for pressing the lever during this phase. If this impulsive lever pressing endures through the delay period into the period of white noise during a no-go trial, the subject will not achieve his goal of obtaining the sucrose reward. Perhaps by inhibiting the dorsal subiculum,

there may be an activation of subcortical structures that may be involved in instrumental behavior responses. The dorsal subiculum has been shown to play a role in movement, and therefore inhibiting the dorsal subiculum may result in increased spontaneous motor activity in situations where it is unfavorable. It was because of this increased lever pressing that the subjects with dorsal subiculum inactivation performed poorly during the no-go trials, due to their inability to withhold from pressing the lever during the period of white noise.

The preliminary data collected from subjects who experienced dorsal subiculum inactivation supports McNaughton's theory that feedback from the subiculum will generally solve response-response conflicts. With the dorsal subiculum inhibited, this may not be possible and an inappropriate response may be initiated (McNaughton, 2006). This coincides with the potential role for the subiculum in response-oriented conflict resolution in a situation where two different responses will result in the achievement of, or failure to reach a specific goal (McNaughton, 2006).

In many ways the experimental paradigm that we established is highly effective for use as a behavioral model of goal-directed behavior. There were, however, several obstacles related to the experimental procedure that we had to combat throughout the course of the experiment. Initially, our surgical technique resulted in compensated structural integrity of the implanted head-holder mount. The region between the skull and the acryloplastic cement presents itself as a prime location for infection, and a few subjects were lost along the way due to infection resulting in the head-holder dismounting from the skull. We developed new techniques during the surgical procedure to reduce the chances of animal loss due to infection/head-holder failure.

We began to use 30% hydrogen peroxide to cauterize the vasculature on the skull and remove any excess tissues that could increase the risk of infection. This greatly increased the structural integrity of the head-holder mount. Prior to this technique, many animals did not make it past the first stages of training. This step was crucial to advancing the success of our experiment because without strong adhesion to the skull, the head-holder mount may detach from the skull while the subject is restrained in the device. The next difficulty for the paradigm we developed is the length of time needed to train each subject to criterion. The entire training process takes several months to complete, and over the course of the training procedure subjects may be lost due to infection or head-holder mount failure. This poses a concern to the researcher due to the value of time that is spent training each animal, however, the power and diversity of the types of data that can be collected during this specific paradigm mor than compensate for the amount of time needed to train each animal.

The current finding of this experiment warrant the need for continued research in order to obtain a larger, more conclusive data set utilizing a larger sample size and more training sessions within each experimental condition. We have demonstrated the utility of the newly developed behavioral task in preparing subjects for pharmacological inactivation of regions of the brain implicated in the brain motivation/reward circuitry. We have also provided preliminary data suggesting anatomical partitioning of function within the subiculum. The dorsal subiculum appears to be involved in impulsivity, whereas the role of the ventral subiculum in the go/no-go task is unclear. There are currently several other rats implanted with dorsal and ventral subiculum cannulae being trained in the go/no-go task. We will be

pharmacologically inactivating these brain regions in the coming months and pooling data from subjects with dorsal and ventral subiculum cannulae, respectively, in order to develop a more conclusive understanding about the roles of each anatomical region in the newly developed reward-related working memory task.

Another facet of this experiment focuses on taking extracellular recordings in the dorsal and ventral subiculum. There are several subjects currently being trained that will be used for extracellular recording in the dorsal and ventral subiculum in the near future. It has been proposed that output mode transitions (bursting to single-spiking) may be needed for proper routing of information in and out of the vSUB and psychostimulants disrupt these changes. This may lead to inappropriate dopamine/novelty signaling that is necessary for setting motivational states and behavior (Cooper et al., 2005). By recording *in vivo* in behaving subjects, it may be possible to correlate these output mode transitions, or simply neuronal firing patterns, to specific aspects of this reward-related working memory task. Beginning by studying how inactivation of the dorsal and ventral subiculum affects behavior, we are able to develop hypotheses about the neuronal firing patterns and their significance within the extracellular recording sessions. The goal of our ongoing, and future, research is to develop a more comprehensive understanding of the partitioned functional roles of the subiculum, which will help to fill the gap in the current literature relevant to the brain motivation/reward circuitry. We believe this will provide valuable insight into the maladaptive plasticity that is associated with motivational and impulse control disorders.

References

Andrzejewski ME, Spencer RC, Kelley AE. Dissociating ventral and dorsal subicular dopamine D_1 receptor involvement in instrumental learning, spontaneous motor behavior, and motivation. Behavioral Neuroscience 2006;**120**(3):542-553.

Cooper DC, Chung S, Spruston N. Output mode transitions are controlled by prolonged inactivation of sodium channels in pyramidal neurons of subiculum. PLoS Biology 2005;**3**:1123-1129.

Cooper DC, Klipec WD, Fowler MA, Ozkan ED. A role for the subiculum in the brain motivation/reward circuitry. Behavioral Brain Research 2006;**174**:225-231.

Cooper DC, Moore SJ, Staff NP, Spruston N. Psychostimulant-induced plasticity of intrinsic neuronal excitability in the ventral subiculum. The Journal of Neuroscience 2003;**23**(30):9937-9946.

Cooper, DC. The significance of action potential bursting in the brain reward circuit. Neurochemistry International 2002;**41**;333-340.

Deadwyler SA, Hampson RE. Differential but complimentary mnemonic functions of the hippocampus and subiculum. Neuron 2004;**42**:465-476.

Hains AB, Arnsten AT. Cognition: Neuropharmacology. Brain and Mind 2009;**1**:1087-1094.

Hampson RE, Deadwyler SA. Temporal firing characteristics and the strategic role of subicular neurons in short-term memory. Hippocampus 2003;**13**:529-541.

Kumari V, Barkataki I, Goswami S, Flora S, Das M, Taylor P. Dysfunctional, but not functional, impulsivity is associated with a history of seriously violent behavior and reduced orbitofrontal and hippocampal volumes in schizophrenia. Psychiatry Research: Neuroimaging 2009;**173**:39-44.

Martin PD. Locomotion towards a goal alters the synchronous firing of neurons recorded simultaneously in the subiculum and nucleus accumbens of rats. Behavioral Brain Research 2001;**124**(1):19-28.

McNaughton N. The role of the subiculum within the behavioural inhibition system. Behavioural Brain Research 2006;**174**:232-250.

O'Mara S, Commins S, Anderson M, Gigg J. The subiculum: a review of form, physiology, and function. Progress in Neurobiology 2001;**64**:129-155.

O'Mara S. Integrating the subiculum into hippocampal formation circuitry and the control of instrumental behavior: theoretical comment on Andrzejewski, Spencer, and Kelley (2006). Behavioral Neuroscience 2006;**120**(3):739-743.

O'Mara SM, Sanchez-Vivez MV, Brotons-Mas JR, O'Hare E. Roles for the subiculum in spatial information processing, memory, motivation, and the temporal control of behaviour. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2009;**33**:782-790.

O'Mara S. The subiculum: what it does, what it might do, and what neuroanatomy has yet to tell us. J. Anat. 2005;**207**:271-282.

Potvin O, Lemay F, Dion M, Corado G, Dore FY, Goulet S. Contribution of the dorsal subiculum to memory for temporal order and novelty detection using objects, odors, or spatial locations in the rat. Neurobiology of Learning and Memory 2009;**93**(2010):330-336.

Segal M. Hippocampal unit responses to perforant path stimulation. Exp. Neurol 1972;**35**:541-546.

Winstanley, CA. The utility of rat models of impulsivity in developing pharmacotherapies for impulse control disorders. British Journal of Pharmacology 2011;**164**:1301-1321.