EFFECTS OF STRIATAL LESIONS ON REWARD CHOICE USING A MULTI-BOX ENVIRONMENT

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ABSTRACT

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Making decisions based on rewards is a behavior that all animals have in common. Being able to decide between an advantageous and disadvantageous choice is an element that could be key to an animal’s survival. While much work has been done investigating which brain areas play a role in this function, there are still many components of reward choice that need to be worked out. We used a novel, 3-box setup to test reward choice in rats. Rats were tested over two, three-week periods where the amount of reward being delivered changed on a week to week basis. We expected choice preference to shift from a mixed-outcome box in the first week, to equal preference in the second week, to the single-outcome box in the third week. This same pattern was to hold true for the second three-week period, except preference would shift according to the changing values in the mixed-outcome box. Preliminary data using 0/2 pellets in a mixed-outcome box showed that rats quickly shifted preference to a 1-pellet reward as opposed to showing equal preference, so a 0/3 pellet mixed-outcome was used instead. During the first three-week session, rats chose between a delivery of 0/3 pellets versus 0 pellets, then 1 pellet, and 2 pellets. Over the second three-week session, rats chose between 1 pellet versus 0/5 pellets, 0/3 pellets, and 0/1 pellet. Rats that received sham lesions showed choice preferences that very closely mapped on to what was expected, showing that they were able to make advantageous choices. To investigate which brain regions may be playing a role in the reward choice process, we performed lesion surgery targeting the ventral striatum, dorsal striatum, or gave rats a sham lesion. While there were not many overall significant group differences, rats with ventral striatal lesions did show a more impulsive choice in week 4 when they preferred the 1-pellet reward opposed to the 0/5 pellet reward. Implementing our new 3-box paradigm in future studies could
help clarify how these brain regions function or malfunction in clinical disorders ranging from addiction to depression.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter/Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAPTER I: INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Choice behavior in the rodent model</td>
<td>1</td>
</tr>
<tr>
<td>Basal ganglia: Anatomy and function</td>
<td>5</td>
</tr>
<tr>
<td>Striatum</td>
<td>7</td>
</tr>
<tr>
<td>Dorsal Striatum and Habit Formation</td>
<td>8</td>
</tr>
<tr>
<td>Dorsal Striatum and Motivation</td>
<td>9</td>
</tr>
<tr>
<td>Ventral Striatum and Reward</td>
<td>11</td>
</tr>
<tr>
<td>Ventral Striatum and Work / Effort</td>
<td>13</td>
</tr>
<tr>
<td>Ultrasonic Vocalizations</td>
<td>14</td>
</tr>
<tr>
<td>CHAPTER II: SPECIFIC AIMS</td>
<td>18</td>
</tr>
<tr>
<td>Aim 1: Choice Making by Rats in a 3-Box Setup</td>
<td>18</td>
</tr>
<tr>
<td>Aim 2: Effects of Striatal Lesions on Choice Making</td>
<td>19</td>
</tr>
<tr>
<td>CHAPTER III: METHODS</td>
<td>22</td>
</tr>
<tr>
<td>Subjects</td>
<td>22</td>
</tr>
<tr>
<td>Surgery</td>
<td>23</td>
</tr>
<tr>
<td>Recovery</td>
<td>24</td>
</tr>
<tr>
<td>Equipment</td>
<td>24</td>
</tr>
<tr>
<td>3-Box Setup</td>
<td>24</td>
</tr>
<tr>
<td>Behavioral Training</td>
<td>25</td>
</tr>
<tr>
<td>Behavioral Testing</td>
<td>26</td>
</tr>
<tr>
<td>Histology</td>
<td>27</td>
</tr>
</tbody>
</table>
CHAPTER IV: RESULTS

Statistical Analyses

Sham Group

Training

Trials

Choice Behavior During Open Testing

Total Pellets Consumed

Total Time in Box

Box Bouts

Entries

Latencies

Food Cup Checks

Ultrasonic Vocalizations

Relative Reward / Choice Discrimination

Total Pellets Consumed

Total time in box

Box Bouts

Entries

Latencies

Food Cup Checks

Ultrasonic Vocalizations

Choice Behavior During the First Ten Minutes of Testing

Trials
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The Three Box Setup</td>
<td>71</td>
</tr>
<tr>
<td>2. The Predicted Star Model</td>
<td>72</td>
</tr>
<tr>
<td>3. Total Pellets Consumed Over 1st Three Weeks (Sham Only)</td>
<td>73</td>
</tr>
<tr>
<td>4. Total Pellets Consumed Over 2nd Three Weeks (Sham Only)</td>
<td>74</td>
</tr>
<tr>
<td>5. Relative Reward / Choice Discrimination Over 1st Three Weeks (Sham Only)</td>
<td>75</td>
</tr>
<tr>
<td>6. Relative Reward / Choice Discrimination Over 2nd Three Weeks (Sham Only)</td>
<td>76</td>
</tr>
<tr>
<td>7. Choice Discrimination Over 1st Three Weeks</td>
<td>77</td>
</tr>
<tr>
<td>8. Relative Reward Effect Over 1st Three Weeks</td>
<td>78</td>
</tr>
<tr>
<td>9. Relative Reward Over 2nd Three Weeks</td>
<td>79</td>
</tr>
<tr>
<td>10. Choice Discrimination Over 2nd Three Weeks</td>
<td>80</td>
</tr>
<tr>
<td>11. Mean Latencies Over 1st Three Weeks</td>
<td>81</td>
</tr>
<tr>
<td>12. Latencies in Single-Outcome Box (W1 – W3)</td>
<td>82</td>
</tr>
<tr>
<td>13. FC Checks During First 10 Minutes (W1 – W3)</td>
<td>83</td>
</tr>
<tr>
<td>14. 50 kHz Ultrasonic Vocalizations Emitted</td>
<td>84</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Weekly Pellet Schedule</td>
<td>17</td>
</tr>
<tr>
<td>2. Weekly Testing Schedule</td>
<td>27</td>
</tr>
</tbody>
</table>
CHAPTER I: INTRODUCTION

Searching for rewards and making choices are behaviors that all animals have in common. Deciding between multiple choices and establishing which is more advantageous is a complex behavior that is essential for an animal’s survival. While it has clearly been shown that more highly evolved animals, such as humans and non-human primates, are able to evaluate rewards (Schultz, Apicella, Scarnati, & Ljungberg, 1992) numerous studies have also shown that rats are capable of making choices and evaluating rewards as well (Cousins & Salamone, 1994; Crespi, 1942; Kearns & Gomez-Serrano, 2011). Performing lesion surgery on specific areas of the brain can help clarify some of the underlying brain functions that are responsible for these behaviors. The goal of the current project is to examine what role the ventral and dorsal subsections of the striatum play in reward choice and decision-making while implementing a novel, 3-box paradigm. Understanding why and how certain choices are made, along with the effects lesions may have on these choices can benefit scientists in many realms ranging from the areas of clinical psychology to neuroscience. Completion of this project could help expand our knowledge of factors involved in choice behavior and possibly lead to more progress in the understanding of mental illnesses such as addiction and impulsivity.

Choice behavior in the rodent model

Although rewards are associated with positive affect, there are times when multiple rewards are available, and the animal could be forced to pick among them, which may lead to a state of decision-making in the animal. When it comes to reward choices, making a decision results from the combination of the previously used actions of the animal and the situation that the animal is currently in (Sutton & Barto, 1998). Careful evaluations of these variables will lead
to the choice of the best possible value of reward. Rewards gain or lose value when compared to other rewards, showing that the intrinsic characteristics of the reward alone are not always the only factor being evaluated. Making a decision based on values of rewards and having a discriminative action to each outcome depending on their values is known as the relative reward effect (also, incentive relativity) (Flaherty, 1996). A classic example of the relative reward effect was shown in an experiment done by Crespi (1942). In this experiment, rats could run down an alley for a large or small reward. Rats that were accustomed to receiving the large reward began receiving the small reward, and when this occurred, their running speed decreased. These particular rats were running at a speed slower than the rats that received only the small reward from the start. The decrease in running speed implies the rats’ ability to judge reward and assign it value. After receiving a reward less valuable than the one previously received, the value of the small reward seems to decrease more than it would have if it were the only reward ever received. This study led to an expansion of studies looking into this effect. One way the expansion has been completed is by looking at how factors such as delay, risk, or reward magnitude will affect the decision being made by an animal.

Craft and colleagues (2011) performed a study in which animals had to evaluate which choice would maintain their overall fitness better: a constant reward of average incentive value or a mixed reward that could be either large or small. They evaluated risk sensitivity in rats based on the daily caloric energy budget (DEB) theory, which assumes an animal will make a food choice based on how much energy (in calories) they will receive from the choice made. In this study, rats were assorted into a grain group and a sugar group. The rats were placed in an operant box and presented with two levers. One lever would reward the rat with 3 pellets of either grain or sugar, while the other lever would randomly reward the rat with either 1 pellet or 5 pellets of
the same product. The authors’ hypothesis was that if a rat received the reward that provided them with more caloric energy, then they would be less likely to take a risk because there would be less of a benefit if they were to receive a smaller reward. In this case, the sugar pellet provided more energy because it was higher in calories. The findings supported their hypothesis. The rats in the sugar group chose to lever press for the mixed reward less often than the rats in the grain group. The authors explain these findings by stating that they coincide with the DEB theory. Since the grain provided less energy, the rats’ overall fitness would decrease. This would lead the rats to be more willing to make a riskier choice in hopes of receiving the larger reward.

The findings of the Craft et al. study may be difficult to interpret. First, the rats in this study underwent an extensive amount of training that totaled up to 21 days. It has been shown that extensive training may lead to actions shifting from being goal-directed to being more of a habit (Adams, 1982). Our study will require a very limited amount of training. This simple change may keep the animal’s subjective value of reward from becoming insensitive to devaluation. Also, the authors state themselves that the rats may have been responding to the sweetness of the sugar compared to the blandness of the grain. This would be a large confound that could completely change the interpretation of their results, as the rats may be viewing the sweetness of the sugar as the reward instead of the caloric intake of the pellet as the reward. Our study controls for this by using one type of sugar pellet. Finally, they attempt to attribute their study to the choices rats make naturally while foraging. The proposed study will draw this comparison even more closely because, as in nature, rats will not be required to press a lever for food delivery. When they enter an operant box, the reward will automatically be delivered. Many studies have been done examining the effects of risk, magnitude, and probability of delivery (Bower, 1961; Cocker, Dinelle, Kornelson, Sossi, & Winstanley, 2012; Lopez-Paniagua &
Seger, 2013), but observing behavior alone is only one way to examine how choices and rewards are evaluated. Studies with a more neurological base take an in-depth look into the underlying mechanisms that mediate choice.

Choices can be altered based on manipulations to different areas of the brain. Cousins and Salamone (1994) performed a study to observe how manipulating activity in the nucleus accumbens altered choice. They gave rats a choice of obtaining the more-preferred 45 mg food pellets by pressing a lever on a fixed-ratio 5 schedule or eating standard chow that was on the floor of the chamber on days 1, 3, and 5 of the experiment. On days 2 and 4 of the experiment, chow was not available. The rats typically chose to press the lever at high rates to obtain the pellets. The rats were then injected with 6-hydroxydopamine, a neurotoxic agent that caused dopamine depletion, in the nucleus accumbens. The rats then began to increase their chow consumption and decrease their lever pressing on days 1, 3, and 5, but lever pressing did not decrease on days 2 and 4. They were led to believe that dopamine depletion in the nucleus accumbens can shift food choice responses, without altering basic behavioral output. These results illustrate that altering activity in this area of the brain can disrupt choice behavior, and in this case, it was shown by the originally desired choice losing value based on the amount of effort needed to obtain it. Combining behavioral and neurological methods in animal models has allowed scientists to gain more information about how choices are made and how choice behavior can be disrupted.

The current project will expand upon past research by introducing a new procedure to evaluate choices made by rats. To the best of our knowledge, most other studies involving rat choice are done in either a runway track, t-maze, or an operant box. New paradigms are still being developed to help find better and more efficient ways to measure choice behavior
This study is expanding upon how this can be measured by broadening the environment for the rat. A three-box setup is going to be used that will allow for an open environment that will more closely resemble the natural world and permit the rats to explore. The three box set-up includes two operant boxes connected to an empty middle box. In this type of environment, the rat will be able to explore more than it would if in a standard operant chamber. A procedure such as the current one requires less training. Often times, too much training may reduce an animal’s dependence on reward value used to make choices, but it will respond out of habit instead (Adams, 1982). For the current task, the rats merely have to enter a box, and the rewards will be dispensed. This, in turn, allows for a more passive role to be played by the rat, and requires it to only learn which reward comes with which box. The rat will need little experience, and no extra responses will need to be learned. This paradigm also allows for more independent measures to be observed at one time, such as choice preference, latencies, ultrasonic vocalizations (USVs), and amount of time spent in each box. Choice behaviors will be observed, and brain manipulations will be completed in this study. The following will provide a more extensive background on the brain and its role in reward choice.

**Basal ganglia: Anatomy and function**

According to Kelley and Berridge (2002), the brain has evolved to decide what choices are appropriate based on diverse variables, and correctly doing this makes for a better chance of survival. The basal ganglia are a group of nuclei in the forebrain that are known for playing a large role in choice behavior. There is still much to be discovered about the basal ganglia due to its extensive connections (Utter & Basso, 2006). The basal ganglia are essential in motor and habit learning (Nambu, 2008). Dysfunctions within the basal ganglia often result in movement
disorders such as Parkinson’s Disease and Huntington’s Disease, which are also accompanied with cognitive decline (Utter & Basso, 2006). Parts of the basal ganglia function in learning the value of rewards, a function in which the neurotransmitter dopamine also plays a large role. The basal ganglia are divided into multiple parts, each of which has separable functions.

The main structures of the basal ganglia are the striatum, subthalamic nucleus, globus pallidus, and the substantia nigra (Utter & Basso, 2006). The output nuclei of the basal ganglia are substantia nigra pars reticula and the globus pallidus internal segment (Utter & Basso, 2006). The superior colliculus, thalamus, and pedunculopontine nucleus are innervated by these two output nuclei of the basal ganglia (Utter & Basso, 2006). The two major input nuclei of the basal ganglia are the striatum, which is made up of the caudate and putamen, and the subthalamic nucleus (Utter & Basso 2006). Most of these nuclei are connected with other nuclei throughout the basal ganglia. There is also a large connection between the basal ganglia and areas of the cortex (Nambu, 2008). Input from the cerebral cortex goes to the basal ganglia, through the thalamus, and back to the cortex, occurring mainly in the frontal lobe (Nambu, 2008). This particular circuit is known as the cortico-basal ganglia loop. There are multiple levels of this loop, and they are all separated physically and functionally (Nambu, 2008). The connection from the basal ganglia through the thalamus is similar to that of the connection of the cerebellum through the thalamus in which they each monitor motor activity (Nambu, 2008). There is a connection in the basal ganglia functions of reward choice and motor control. As some studies have shown that the neurotransmitter dopamine can shift reward choice (Cousins & Salamone, 1994), others have shown that dopamine depletion may also disrupt motor control (Chagniel, Robitaille, Lacharité-Mueller, Bureau, & Cyr, 2012). It is important to keep in mind that
dopamine depletion may have differing effects based on which brain area is depleted, as well as the extent at which the depletion occurs.

*Striatum*

The striatum is the main input structure within the basal ganglia, and it plays a large role in determining reward value, yet there is still uncertainty as to how this structure influences reward evaluation in a type of open environment such as the one to be utilized in this study. There are connections to the striatum from almost every part of the cortex (Gerfen, 1992), and there are also inputs from limbic structures such as the amygdala and hippocampus, which has led to the striatum being called the limbic-motor interface (Floresco, 2006). Dopamine inputs arrive from the ventral tegmental area (VTA) (Basar et al., 2010), and the substantia nigra pars compacta (Delgado, 2007). The striatum can be divided into ventral and dorsal sections. The dorsal striatum (DS) is recognized mainly as the caudate nucleus and putamen (Basar et al., 2010) and has the largest amount of dopamine receptors of any area in the brain (Yin, Ostlund, & Balleine, 2008). The ventral striatum (VS) mainly consists of the nucleus accumbens, but also contains the olfactory tubercle and small portions of the caudate nucleus and putamen (Basar et al., 2010). The nucleus accumbens can also be broken down into two areas. First, is the core, which is partially surrounded by the second area, the shell (Kelley, 2004). There is a growing amount of research showing a dissociation in functions between these two areas (Floresco et al., 2006; Corbit et al. 2001; Ito et al., 2004). Generally, the core plays a much more prominent role in the learning of instrumental actions, while the shell plays a large role in anhedonia and the process of extinction (Muschamp et al., 2011). The following sections will give a more in-depth look into these specific areas.
Dorsal Striatum and Habit Formation

The dorsal section of the striatum has been found to play a prominent role in behavioral functions. With a connection to the prefrontal cortex, motor cortex, and sensory cortex, the DS is partially responsible for habit formation (Everitt & Robbins, 2005). Dickinson and colleagues (1995) have shown that habit formation occurs when an animal undergoes an extended amount of training with consistent conditions. According to these authors, when an action becomes a habit, it occurs automatically in response to a stimulus, and it becomes disconnected from any shift in reward value. Yin, Knowlton, and Balleine (2004) performed a study looking into the function of habit formation. Rats were given lesions to the dorsolateral striatum, and then trained to lever-press to get a drink of sucrose reward. After their trials, one group of rats was given intraperitoneal injections of lithium chloride to induce a taste aversion and devalue the reward. Both rats with DS lesions and sham lesions reduced their consumption if they had received the injections. Then, they were ran through an extinction sessions where the lever was presented, but no reward was given for pressing it. Rats that had dorsolateral striatum lesions that also had the reward devalued had a significantly lower rate of pressing the lever than those with sham lesions. The authors use this as evidence that this area of the striatum is necessary for habit formation. By damaging the area of the brain that allows for the process of habit formation, they were able to disrupt the process, leading to a much lower rate of lever pressing. When an action that has been occurring in a relatively automatic fashion fails to persist, it can be concluded that the formation of that habit has been disrupted. This may lead us to believe that if a positive reward is presented to a rat, then lesion to the dorsal section of the striatum may disrupt the stimulus-response habit formation leading up to retrieving the reward.
Along with habit formation, it has been shown that the dorsal striatum is involved in motivation, which could be considered a key aspect in reward choice and decision-making. In order for an animal to perform, it needs to be motivated to do so. The link between motivated behavior and the DS has been studied using dopamine-deficient mice. Mice that lack the gene responsible for the biosynthesis of dopamine become hypophagic, and they will die by the time they reach four weeks of age (Zhou & Palmiter, 1995). Szczypka and others (2001) found that replacing dopamine in the DS of dopamine-deficient mice will motivate them to eat, but restoring it in the VS only motivates them to explore. Ito, Dalley, Robbins, and Everitt (2002) showed that dopamine levels are elevated in the DS of rats while they are seeking cocaine after a cue has been presented.

Studying a subjective aspect such as motivation can be difficult using animal models. Volkow and colleagues (2002) performed a study in humans looking at the connection between the DS and what they termed “nonhedonic motivation”. What this means is that their subjects were able to see and smell their reward, without actually consuming it. It is believed that the VS plays the role in processing the hedonic value of rewards (Kelley & Berridge, 2002). Volkow and colleagues hypothesized that the DS would then mediate the non-pleasurable wanting of the reward. For the experiment, subjects were food-deprived for 16 – 20 hours before testing. They then underwent a PET scan while their favorite foods were presented to them. Extracellular dopamine levels were recorded during this period, and the participants were also asked to rate their hunger levels. It was found that extracellular dopamine levels were increased in the DS during this period, but not in the VS. Also, there was a correlation between the increase in extracellular dopamine and levels of hunger being reported by the participants; the greater the
increase in extracellular dopamine, the greater the desire for the food. Volkow and others (2006) found similar results in a study where they investigated extracellular dopamine levels in the DS in cocaine-addicted individuals. When shown videos of people preparing and using cocaine, the participants had a similar increase in subjective craving and extracellular dopamine in the DS as those in the food study. Once again, there was no increase in extracellular dopamine in the VS. The investigators conclude from these results that dopamine in the DS is related to an increase in motivation to obtain the reward.

A case study reported by Muskens, Schellekens, Leeuw, Tendolkar, and Hepark (2012) revealed similar results. They examined the behavior of a 75-year-old man who had an ischemic stroke. The man had excessively used alcohol and nicotine for twenty years with no desire to stop. After his stroke, he completely ceased use of each of the drugs. An MRI had revealed that the stroke caused a unilateral lesion to the dorsal striatum on the left side of the man’s brain. These results fall in line with the aforementioned studies, indicating that the lesion of the DS in this patient may have eliminated the motivation underlying his prior abuse of alcohol and nicotine. From these studies, we can draw the conclusion that the DS plays a role in motivating animals to make a choice to seek out reward.

These results may be slightly contradicting. When we say that the DS is involved in habit formation, we are referring to a behavior that is mostly automatic. On the other hand, the neuroimaging studies imply that the DS is involved in motivation, a flexible aspect of behavior. These somewhat opposing aspects could possibly be attributed to the findings that the DS is made up of two subsections that are functionally dissociated (Featherstone & McDonald, 2003). The dorsolateral striatum seems to be implicated more in habit formation (Yin, Knowlton, &
Balleine, 2004), while the dorsomedial striatum plays a larger role in motivated behaviors (DiFeliceantonio, Mabrouk, Kennedy, & Berridge, 2012).

Ventral Striatum and Reward

Work done with nonhuman primates has found that the VS is largely active during the state of expecting reward (Cromwell & Schultz, 2003; Schultz, Apicella, Scarnati, & Ljungberg, 1992), decision making in relation to rewards (Wickens et. al. 2007), and determining incentive value (Kelley, 2004). Schultz and colleagues (1992) performed a study examining the firing of neurons in expectation of reward in monkeys. The monkeys were trained in a go-no-go task to receive reward. In this task, either a green or red light was presented to the monkey whose arm was in a fixed position. If the green light appeared, it would be followed by a short yellow light with the presentation of a lever. The yellow light was considered the trigger light, and it was used to produce a state of reward anticipation. The monkey would press the lever to receive a liquid reward, which would be delivered after a short delay (Go). If the red light would appear (No-Go), the monkey would only receive the reward if it did not press the lever. Recordings of neurons in the VS were taken during this task. They found that 43 neurons in the VS were active during the delay period between the lever press and delivery of reward. These results indicate that the VS plays a role in the expectation of reward based on past experiences. Normal functioning in this area of the brain would be essential for an animal to properly determine which reward to expect in a given circumstance. Without this area properly functioning, animals would solely have to rely on impulsive choices. Without the ability to predict what may come, they would not have the opportunity to plan their actions ahead of time.
The nucleus accumbens is the main subsection of the VS. A few functions that the nucleus accumbens plays a role in are updating reward value, effort-based decision making, instrumental learning, impulsivity, addiction, and reward evaluation of natural reinforcers (Cardinal et al., 2001; Hauber & Sommer, 2009; Kelley, 2004). Instrumental learning could be a crucial part in obtaining rewards, but it has been shown that improper functioning of the nucleus accumbens may have a detrimental effect on this when a delay is presented before the delivery of reward. A study examining this behavior was completed by Cardinal and Cheung (2005). They examined the ability of rats to learn instrumental task of lever pressing and act upon it when a delay is placed before the delivery of reward. Rats were placed into an operant box that contained two levers. One lever would give one food pellet, while the other lever had no reward. The one-pellet lever would either deliver the pellet immediately, after a 10-second delay, or after a 20-second delay. Once adequately trained, a group of rats were given nucleus accumbens core lesions. Rats that were given nucleus accumbens core lesions performed significantly worse than sham-lesion rats when a delay occurred before the reward was given.

To test that nucleus accumbens core lesions only had an effect on the rat’s ability to learn when there was a delay and not affect the ability to learn the reward itself, a second experiment was performed. The rats were placed in the same environment, but the function of the levers was changed. One lever would provide one pellet, while the other would provide four. By the end of the experiment, both sham and nucleus accumbens lesion rats preferred the larger magnitude reward. Given these results, the experimenters hypothesized that the nucleus accumbens core serves to “bridge” over the delay time from when the rat commits an act to reward reception. Basically, if animals receive nucleus accumbens core lesions, it has no effect on a rat’s ability to learn about the reward itself, but it does have an effect if a delay occurs before the reward.
Having this type of effect on delayed rewards has led researchers to believe that the nucleus accumbens may have a part in mediating impulsive choices. This could also be a factor that underlies why an animal may show perseveration. Increasing an animal’s impulsivity increases the likelihood that it will choose a reward that will be less valuable, regardless of original hedonic value. This illustrates that proper functioning of the nucleus accumbens is essential for making rational, advantageous choices.

**Ventral Striatum and Work / Effort**

One of the advantages of having the capability to evaluate a reward before its delivery is that it will allow the animal to decide how much effort it wants to expend in order to retrieve that reward. Extensive work has been done on the ventral striatum and the neurochemical dopamine on work effort and evaluating rewards. (Cousins, Sokolowski, & Salamone, 1993; Cousins, Trevitt, Atherton, 1999). There is a large number of dopamine receptors in the striatum, with the largest concentration of them in the brain being in the DS (Yin, Ostlund, & Balleine, 2008). Cousins and colleagues (1993) looked at the effect that dopamine depletion in the ventrolateral striatum has on effort. They trained rats to lever press for a food pellet reward on a FI-30 schedule (only the first lever press after 30 seconds was rewarded). Then the rats underwent surgery where they received bilateral injections of 6-hydroxydopamine (6-OHDA) into either the nucleus accumbens or ventrolateral striatum. Their results showed that when dopamine was depleted in the nucleus accumbens, lever pressing was not significantly affected. They found that lever pressing decreased as a result of dopamine depletion in the ventrolateral striatum. These results show that motivation and effort are reduced with decreased levels of dopamine in the ventrolateral striatum. Cousins et al. (1999) have also shown that dopamine depletions in the nucleus accumbens will significantly lower locomotor activity, as well as have a trending
decrease in locomotor activity when occurring in the ventrolateral striatum. With lesions to these areas, rats may choose a reward that is more easily accessible than the one that may be more beneficial. Findings such as this could also explain why some rats might not show interest in any certain reward. When this area of the brain is being inhibited, the cost of expending energy could outweigh the result of getting a reward.

_Ultrasonic Vocalizations_

The current study is going to look at choice behavior as well as the communication that occurs during it. Rats are capable of communicating in the form of USVs. Panksepp (2000) states that USV calls in the 50 kHz range are a type of call that signifies a positive affective state, and these can be induced by “tickling” the rat, thus causing what he termed rat laughter. This laughter was elicited by tickling rats on the nape of their neck, an action that resembled juvenile play (Panksepp, 2007). Rats also use USV calls in the 22 kHz range to convey a negative affective state (Brudzynski, 2007). In a study looking into this, it was found that when rats received foot shocks, they would vocalize 22 kHz USVs and freeze. Afterwards, when 22 kHz USVs would be played back to them, they froze, thus leading the researchers to believe that the rats were able to relate the sound of 22 kHz USVs to an internal state of fear (Parsana, Moran, & Brown, 2012). USV’s are one way that rats may be able to communicate to each other to signal that they are in either a positive or negative situation.

USVs have also been studied as they are involved in a variety of cases of reward such as electrical brain stimulation, somatosensory hand play, and drug reward. Burgdorf and colleagues (2000) examined the relationship between 50 kHz USVs and reward anticipation. In two of their experiments, they used electrical brain stimulation to either the VTA or the lateral hypothalamus.
every 20 seconds as a reward. They found that rats elicited increased amounts of 50 kHz USVs before they would receive the stimulation. They also found that when the rats were able to self-administer the reward, they would make more 50 kHz USVs when they would see a cue that signaled the opportunity was coming. In each case of reward, experimenter-delivered or self-administered, when the reward would be terminated without cue, the rats would make a 20 kHz USV. In this same study, they also found that rats would increase their rates of 50 kHz USVs when a cue was given that signified a one hour feeding time.

In another study, Burgdorf and colleagues (2001) examined the effects of amphetamine microinjections into the nucleus accumbens, and the effect that this had on USVs. They found that there was a dose-dependent effect on the amount of USVs made, and that amphetamine microinjections made the rats produce more 50 kHz USVs. From their findings, they drew the conclusion that the amphetamine injections increased the amount of dopamine in the nucleus accumbens, thus causing the rat to anticipate reward and vocalize in the form of 50 kHz USVs. The increase in 50 kHz USVs seems to signify not only positive affect, but also a positive state of anticipation or arousal. From this, it can be concluded that rats understand that they are about to receive something that they desire, and USVs can be looked at as a way to communicate this excitement. USVs will be recorded in the present study and will add factors for indicating the emotional state of the animals.

The current project will expand the knowledge of choosing rewards and the neuroscience behind this behavior with contributions from multiple factors. There currently are gaps in the knowledge that have not been filled. One of these gaps is from the spatial and behavioral restriction placed on the animals in standard experiments. The innovative 3-box setup used in the current study will allow for a more open environment that gives them more freedom than a
typical 1-box setup. From this freedom, we will be better able observe and measure a more complete set of behavioral responses. The open environment will also allow for the animal to make more natural responses, as well as responses that have not been corrupted by extensive training.

Originally, we were going to use pellet magnitudes of 0, 1, and 2 pellets versus 0 or 2 pellets over a three-week period. We then added a second three-week period where the pellet magnitude in the mixed-outcome box would change. We expected the sham-lesion group to make choices that resemble our “star” model (see Figure 2). Based off of this model, rats would make optimal choices based off of which box dispensed a greater magnitude of pellets over time. The star model states that in week 1, rats would make most of their choices in the 0 or 2 box. This preference would be almost equal in week 2 due to the magnitudes being equal. Preference would then shift in week 3 to the box with 2 pellets, resembling a pattern that is almost the opposite of that shown in week 1. We expect this same pattern to hold true for weeks 4 through 6, with week 4 being similar to week 1, week 5 being similar to week 2, and week 6 being similar to week 3. Preliminary data showed that there was an extreme shift to the single-outcome box in week 2. Logan (1965) stated that incentive value takes into account both magnitude and delay. In order for a small reward to be preferred over a large reward, there must be a significant amount of time before the large reward is given. From this preliminary data, we could conclude that 0 or 2 pellets with a 5-second delay between deliveries did not have enough incentive value to outweigh 1 pellet being delivered every 5 seconds. This led us to increase the magnitude in the mixed-outcome box. Instead of dispensing 0 or 2 pellets, the mixed-outcome box would dispense 0 or 3 pellets. Table 1 shows the schedule for the changing amount of pellets being dispensed.
<table>
<thead>
<tr>
<th>Week</th>
<th>Pellets in Single-Outcome Box</th>
<th>Pellets in Mixed-Outcome Box</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 pellets</td>
<td>0/3 pellets</td>
</tr>
<tr>
<td>2</td>
<td>1 pellet</td>
<td>0/3 pellets</td>
</tr>
<tr>
<td>3</td>
<td>2 pellets</td>
<td>0/3 pellets</td>
</tr>
<tr>
<td>4</td>
<td>1 pellet</td>
<td>0/5 pellets</td>
</tr>
<tr>
<td>5</td>
<td>1 pellet</td>
<td>0/3 pellets</td>
</tr>
<tr>
<td>6</td>
<td>1 pellet</td>
<td>0/1 pellet</td>
</tr>
</tbody>
</table>

*Table 1. Weekly Pellet Schedule*
CHAPTER II: SPECIFIC AIMS

Aim 1: Choice Making by Rats in a 3-Box Setup

The first aim of this study is to observe and monitor the behavior of rats while they make and evaluate choice. We are going to look at how they value choices relative to others in a paradigm that has not yet been used. USVs will also be recorded to aid in the observations of the rats’ behaviors. We will use the dependent measures of total pellets consumed to judge hunger and motivation. We will measure the total time spent in each box and average time spent in each box as a measure of preference. The total number of food cup checks will be used as a measure of compulsiveness. Latencies to retrieve pellets will be measured to assess motivation as well as motor coordination. 50 kilohertz USVs will be used to measure the positive affect associated with each box.

As we have seen from Crespi’s early work, rats are able to make choices based on magnitude, and with higher magnitude comes a more apparent preference for that reward. Our plan is to use the comparisons: 0 pellets to 0 or 3 pellets, 1 pellet to 0 or 3 pellets, and 2 pellets to 0 or 3 pellets for the first three-week session. For the second three-week session, we plan to use the comparisons: 1 pellet to 0 or 5 pellets, 1 pellet to 0 or 3 pellets, and 1 pellet to 0 or 1 pellet. We will use the first 3-week period to change the pellet reward between weeks in the single-outcome box. During the second 3-week period, the pellet reward will change between weeks in the mixed-outcome box.

This new design allows for multiple measures of reward comparison. The first comparison being measured is choice behavior. Choice behavior will be defined as how rats show preference between the single-outcome box and the mixed-outcome box within a one-week time period. Choice discrimination is defined as the comparing the reward in the box where the
pellet value changes over the three-week period. This refers to the single-outcome box during the first three-week period and the mixed-outcome box during the second three-week period. Finally, relative reward is defined as comparing the reward that does not change between weeks during each three-week period. This refers to the mixed-outcome box during the first three-week period and the single-outcome box during the second three-week period.

**Aim 2: Effects of Striatal Lesions on Choice Making**

The second aim of this study is to gain more knowledge on how the brain is involved in the rats’ choices of rewards. We plan to lesion the VS or DS and compare the effects to sham lesions. We expect the control rats to exhibit behaviors similar to those as mentioned in the previous section. The main differences we expect to see come from the dorsal and ventral striatum lesions.

Multiple studies lead us to believe that we can expect to see choice preference altered when animals receive DS lesions (Chagniel et al., 2012; Gengler, et.al. 2005; Volkow, et.al. 2002). We will have to monitor motor movements within the dorsal lesion group due to possible motor deficits that may be caused (Chagniel, et.al. 2012). With DS lesions, we may expect to see difficulty with movements. This could cause the rat to have difficulty navigating the environment and making advantageous choices. This could affect the amount of times a rat is able to enter a box or the time it takes to receive a pellet after it is dispensed (latency).

We can also expect to see a decrease in the amount of habit-like behaviors in the animals with DS lesions. Yin, Knowlton, and Balleine (2004) showed that habit formation is disrupted with dorsal striatum lesions. With the previous evidence, we hypothesize that the rats will show
less compulsive food cup checking. In other words, their habit of consistently checking the food cups will be disrupted. Consequently, this would lead the overall number of trials performed to decrease.

Previous work has shown us that the VS plays a role in impulsivity (Cardinal, Pennicott, Sugathapala, Robbins, & Everit, 2001). Impulsivity can be defined as an inability to wait for reward. In the current paradigm, there is a shorter amount of time that the animal would have to wait between the constant rewards than there would be in the mixed 0 or 2 pellet box. For example, there is always a 5 second delay between rewards when they are constant, but the 0 or 3 pellet reward can sometimes result in two consecutive trials of 0 pellets being delivered, which would result in a 15 second delay between deliveries of the 3-pellet reward. Impulsivity could lead the rat to prefer the box where this extended delay would not occur.

We also need to keep in mind that Cardinal and Cheung (2005) have shown that proper functioning of the VS is needed for rats to learn about a reward when there is a delay. As mentioned before, the 0 or 3 pellet reward may result in a 10 – 15 second delay period. This may be too long of a span for the rats with ventral striatal lesions to be able to “bridge” over. These findings leads us to believe that rats with VS lesions will show a more distinct box preference for the boxes that give the constant reward, but only in the final two weeks of the experiment. With this in mind, we can expect that the rats with VS lesions may show no difference in behavior from the sham control group in week 1, but they may begin to persist on the 1 and 2 pellet reward boxes in weeks two and three due to the shorter delay period occurring before reward delivery. We expect these rats to show a similar pattern of box preferences as the one shown by the sham group, but it will be more extreme. In other words, we expect them to have an even stronger preference for the 1 and 2 pellet reward boxes than the shams, while also having very
little interest shown for the 0 or 3 pellet reward box. This could be shown quantitatively by an increase in trials performed, food cup checks, or the amount of time spent in the box delivering the constant reward while also having a decrease in these measures in the 0 or 3 pellet reward box.
CHAPTER III: METHODS

This experiment will require the use of rats since the nature of the study is too invasive for humans. Rats will undergo surgery where they will receive a lesion to the DS, the VS, or a sham lesion. There will be three groups of 10 - 12 rats for each lesion. Rats will be tested over a period of six weeks in a three-box paradigm. The two boxes connected to the middle box will have different magnitudes of pellets being delivered, and the rat will have the choice of which box to stay in. For the first three-week session, one of the boxes will dispense a fixed amount of pellets or no pellets (0 on the first week, 1 during the following week, and 2 during the final week), and the other will dispense a mixed amount of pellets (0 or 3). For the second three-week session, the single-outcome box will dispense one pellet all three weeks, while the mixed-outcome box will change over the course of the three weeks (0 or 5 pellets during week 4, 0 or 3 pellets during week 5, and 0 or 1 pellet during week 6). The single-outcome box and the mixed-outcome box will be counterbalanced between animals to avoid any spatial preference. Each week will consist of five days of testing. The first two days will be training, the next two days will be “open” days, and the final day will be the extinction day. USVs will also be recorded on the final two days of the experiment. Rats will have *ad lib* access to food from the time they are returned to their home cage on day 5 until the Sunday before they are tested again. After the six-week testing period, the rats will undergo perfusion, and brain tissue will be observed to locate areas of the lesion. For visual representation of the 3 box set up, see *Figure 1*.

*Subjects*

32 male Sprague-dawley rats (Rattus norvegicus) will be used throughout the course of this experiment. Their weight will be taken for three days prior to the experiment and averaged
to gain a baseline. 85% of their baseline weight will be used as their target weight to ensure the health of the animals. If they drop below 85% of their original baseline, they will be removed from the experiment. All subjects will be housed individually in 65 X 24 X 15 cm cages. They will be food deprived for the week of the experiment, but we will try to keep their weight around 90% of the baseline. They will have *ad libitum* access to water.

**Surgery**

Surgery will be conducted according to sterile procedures. Surgical instruments will be sterilized using an autoclave or dry bead sterilizer. Animals will be anesthetized with isofluorane using a small animal anesthesia system. General anesthesia is maintained throughout the operation, and signs of anesthesia include loss of sensory reactivity and slowed respiration rate. A stereotaxic apparatus will be used to target lesions into VS (A +0.7 – 1.2, M ± 0.7 – 1.2, D -7.0 – 7.5 mm relative to bregma) or DS (A +0.7 – 1.2, M ± 2.9, D -4.7 mm relative to bregma) according to the standard rat stereotaxic atlas (Paxinos & Watson, 1997). After anesthesia, the dorsal head region will be cleaned, shaved, and retracted to reveal the skull surface. Once stereotaxic position is obtained, a small craniotomy will be completed above the target site (2 for each animal as each lesion will be bilateral). A microsyringe will be lowered to the exact location of the brain region. The syringe will contain either the neurotoxin 0.09 M quinolinic acid dissolved in 0.1 M phosphate buffer (vehicle), with pH adjusted to 7.4 using 0.1 M NaOH or vehicle (PBS alone). Bilateral infusions will be made via 31 gauge stainless-steel injector attached to a Hamilton microinfusion pump by polyethylene tubing according to the following parameters: (a selective neurotoxin) or phosphate buffered saline (sham lesion). The volume will be 0.5 microliters and the time for infusion will be 3 – 5 minutes. After infusion into the left and right sides, the holes will be filled with gel foam and the skin sutured. Animals will be taken out
of the stereotaxic device and observed. After surgery, rats will be allowed time to recover (7 – 10 days) prior to commencement of testing.

Recovery

Immediately following surgery, animals will be monitored continuously. Body temperature, heart rate, respiratory rate, and general condition will be recorded in post-surgical recovery logs at ten minute intervals. Fluids and heat will be provided as necessary. As the animal recovers, it will be monitored every 6 – 10 hours. Animals will be kept warm and dry, and the need for analgesic medication in individual animals will be based on the animal’s behavior. General disposition, level of activity, whether the animal is eating and drinking, and vocalizations when the animal is handled will be used to assess post-operative need for analgesic medication. Beginning 24 hours after surgery, recovery of the animals will be monitored every 24 hours for post-surgical complications such as infections, changes in behavior, inappetence, lethargy, or other indicators of distress. Animals that display problems will be monitored more frequently (every 20 minutes to every 6 hours, as needed, depending on the severity of the problem). University Animal Facilities staff and the Attending Veterinarian will be notified immediately in the event of abnormal post-surgical recovery.

Equipment

3-Box Setup. The three box set up is a novel and untested design. It will consist of two standard operant boxes (Med Associates, 10 X 12 X 16 inches) connected to a box of the same size by acrylic sheet tubing. The operant boxes contain a food receptacle directly across from the entry area of the tube, a lever, and a place for a water nozzle. They are located inside two sound-attenuating chambers. The middle box is not encased by a sound-attenuating chamber. Cameras
are located above the two operant boxes to observe the rats’ behaviors. USV detectors are placed on top of the operant box with the receiver pointed towards a small set of holes that allow for easier USV pickup. A white noise generator is used to cancel out any noise that may interfere with the USV recordings. This generator is needed because the sound attenuating chambers are not completely soundproof. Without the white noise generator, sounds such as experimenter noise may interfere with the recording of USVs. It will also be used to equate the auditory experience for each animal over all sessions. The two operant boxes are connected to a computer using the MED-PC program for data collection and running the program. The MED-PC software has been programmed to specifically measure all of the dependent variables being investigated in this study.

**Behavioral Training**

For this experiment, behavioral training consists of the first two days that the rats are placed in the 3-box set up. The purpose of this behavioral training is to teach the rat which reward magnitude is associated with each box. This also serves to keep from overexposing the rats to operant behaviors. Once the Med-PC program is started, the rat will be placed in the middle box. It will have to make a choice as to enter either the box to the left (Box 1) or the box to the right (Box 2). An infrared beam is located just before the entrance of the box, and once it is broken, a guillotine door will lower, trapping the rat in that box for ten minutes. A pellet will then be dispensed into the food cup after a five-second delay. After five seconds if the infrared beam in the food cup is broken (the pellets are retrieved), more pellets will be delivered. Pellets are only delivered if the beam is broken after the five-second delay. By containing the rat in one box for ten minutes on training days, there should not be any confusion that could occur by allowing it to consistently switch between boxes when it is new to each. After 10 minutes, the
guillotine door will open allowing the rat to exit the box, and proceed toward the opposite box. An infrared beam is located before the center box that, when broken, will lower the guillotine door behind the rat to prevent it from re-entering the initial box. The same process will occur in the opposite box once the rat reaches it, with the only difference being the pellet magnitude being delivered.

This novel training paradigm creates multiple advantages over the traditional one-box system. By allowing the rat to explore during training as opposed to teaching it to respond to something for a reward, it will more closely mimic a natural environment. Also, extensive training may lead to habit formation, and this could cause the animal to value the reward less (Adams, 1982).

**Behavioral Testing**

The testing period is very similar to the *Behavioral Training* period. It will occur for two days immediately after the initial training days. The rat will be placed in the middle box and permitted to roam through the environment. For this period, though, the procedure will not stop before the thirty minute time period has expired. Also, the rat will not be trapped in the box once it makes a choice. It will be free to roam all three boxes for the entire thirty minutes. The rat will have *ad libitum* access to water for the duration of the experiment. During the thirty minutes, time spent in each box, food cup latencies, 50 kHz USVs, pellets not eaten, amount of water drank (in grams), and the amount of times each food cup is checked will be recorded. Lever presses will also be recorded to measure superstitious behaviors, but pressing the lever is not reinforced. On the final day of the testing period, food pellets will be removed so the rat will not
be able to gain any reward. This will be the extinction day. Other than the lack of pellets, all other parameters will remain the same.

<table>
<thead>
<tr>
<th>Behavioral Training</th>
<th>Monday – Tuesday</th>
<th>2, 20-Minute Sessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavioral (Open) Testing</td>
<td>Wednesday – Thursday</td>
<td>30-Minutes</td>
</tr>
<tr>
<td>Extinction</td>
<td>Friday</td>
<td>30-Minutes</td>
</tr>
</tbody>
</table>

*Table 2. Weekly Testing Schedule*

**Histology**

After all testing has been completed, rats will be euthanized using 100 mg/kg of sodium pentobarbital-based solution. An intracardial perfusion will then be performed using 0.9% saline solution followed by 10% formalin in PBS. The brain will then be removed and stored in a 30% sucrose solution (10% formalin) for a period of 2 - 4 days, or until it sinks. The brain will then be blocked, frozen, and sliced in 30 μm slices using a sliding microtome. Slices will be fixed to a slide and stained using cresyl-violet. The slides will be analyzed to verify that lesions occurred in the intended areas.
CHAPTER IV: RESULTS

Statistical Analyses

We ran all statistical analyses using IBM SPSS Statistics 20 (Armonk, NY). We used Kolmogorov-Smirnov tests to test the assumption of normality. We tested all variables, and only about 15% (18 of 96) of the Kolmogorov-Smirnov tests were significant. This led us to use parametric tests. We used an analysis of variance (ANOVA) to test for significance for all data. In any instance that the assumption of sphericity was violated, we reported the significance using the Greenhouse-Geisser correction. We followed up any significant findings obtained from the ANOVAs using pairwise t-tests when looking at data from only the sham lesion group. For between group comparisons, we used Fisher’s Least Significant Difference (LSD) tests. No post-hoc corrections were applied to the t-tests (see O’Keefe, 2003). P-values less than .05 were considered significant.

Due to technological difficulties involving faulty connections, data analyzed for ultrasonic vocalizations contains only 18 of the 31 rats tested. The problem arose from multiple instances of equipment failure. There were 5 rats in the sham group, 7 rats in the dorsal lesion group, and 6 rats in the ventral lesion group. This complication could have an effect on the power of the USV analysis.

Sham Group

Training

Trials. Trials from Training Day 2 were analyzed as an indicator of preference for each week (W). A 2x3 factorial ANOVA of the first three weeks revealed a main effect of box, $F(1,8) = .342, p < .05$. Pairwise t-tests revealed a significant difference between boxes in W1 and W3(W1: 0 pellets < 0/3 pellets; $M = 12.11± 3.37$ vs. 30.44 ± 5.63 trials, $t(8) = -2.46, p < .05$;
W3: 2 pellets > 0/3 pellets; \( M = 51.22 \pm 5.7 \) vs. \( 37.89 \pm 5.2 \) trials, \( t(8) = 2.33, p < .05 \). This pattern follows our proposed model of choice behavior for sham rats, which shows that they indicate preference by the number of trials they have completed in each box.

A 2x3 factorial ANOVA of trials during Training Day 2 for the second three weeks found a main effect of box (\( F(1,7) = 31.56, p < .01 \)) and a main effect of week, \( F(2,14) = 25.27, p < .01 \). Results of pairwise t-tests found significant differences in box choice in W4 and W6 (W4: 1 pellet > 0/5 pellets; \( M = 47.44 \pm 6.9 \) vs. \( 16.78 \pm 4.04 \) trials, \( t(8) = 3.46, p < .01 \); W6: 1 pellet > 0/1 pellet; \( M = 85.88 \pm 3.38 \) vs. \( 44.12 \pm 7.06 \) trials, \( t(7) = 4.92, p < .01 \)). Given that the mixed-outcome box in W4 provides the highest overall magnitude, the results of W4 may be reflecting an aversion to delayed reward.

**Choice Behavior During Open Testing**

*Total pellets consumed.* To assess choice behavior, we analyzed data between boxes over each 3-week testing period. This is data taken from the overall, 30-minute session. The total amount of pellets consumed was calculated from multiplying the number of trials the rat performed in each box by the magnitude of reward that was dispensed in that box per trial, and then subtracting the amount of pellets the rat did not consume by the end of that session. Over the course of the first three weeks, we found a main effect of week, \( F (2,16) = 5.70, p < .05 \), as well as a box by week interaction, \( F (2,16) = 31.32, p < .01 \). Pairwise t-tests revealed significant differences between boxes in week 1 and week 3 (W1: 0 pellets < 0/3 pellets; \( M = 0 \pm 0 \) vs. \( 185.5 \pm 21.4 \) pellets, \( t(8) = -8.65, p < .01 \); W3: 2 pellets > 0/3 pellets; \( M = 191.3 \pm 25.1 \) vs. \( 53.4 \pm 15.7 \) pellets, \( t(8) = 4.35, p < .01 \); see Figure 3). This pattern of results also resembles the proposed model for control animals.
A 2x3 factorial ANOVA of the second three week session revealed a box by week interaction, $F(2,16) = 17.35$, $p < .01$. Pairwise t-test found a significant difference in box choice in W6 (1 pellet > 0/1 pellets; $M = 186.56 \pm 18.53$ vs. $11.89 \pm 2.61$ pellets, $t(8) = 8.88$, $p < .01$; see Figure 4).

Total time in box. The total amount of time the subjects spend in each box is recorded (in seconds) as an indicator of how much value the rat assigns to the reward in that box. We can expect that if a rat spends more time in a specific box, then it has assigned a higher value to the reward in that box. A 2x3 factorial ANOVA of the first three week session revealed a box by week interaction $F(2,16) = 20.69$, $p < .01$. Pairwise t-tests found a significant difference between boxes in W1 (0 pellets < 0/3 pellets: $M = 289.12 \pm 32.71$ vs. $1075.00 \pm 83.62$ seconds, $t(8) = -7.51$, $p < .01$).

A 2x3 factorial ANOVA of the second three weeks session found a box by week interaction, $F(2,16) = 9.03$, $p < .01$. There was a trending effect of box, $F(1,8) = 4.33$, $p = .071$. Pairwise t-tests found a significant difference between boxes in W6 (1 pellet > 0/1 pellets: $M = 1106.75 \pm 84.17$ vs. $306.44 \pm 49.13$ seconds, $t(8) = 6.64$, $p < .01$).

Box bouts. Box bouts refers to the average amount of time (in seconds) a rat spends in a box each time it makes the choice to enter it. A 2x3 factorial ANOVA of the first three-week session revealed a box by week main effect, $F(2,16) = 8.78$, $p < .01$. Pairwise t-tests found a significant difference between boxes in W1 (0 pellets < 0/3 pellets: $M = 22.18 \pm 2.89$ vs. $66.53 \pm 7.49$ seconds, $t(8) = -7.24$, $p < .01$).

A 2x3 factorial ANOVA of the second three-week session found a box by week interaction, $F(2,16) = 6.68$, $p < .01$. Pairwise t-tests found a significant difference between boxes in W6 (1 pellet > 0/1 pellets: $M = 81.61 \pm 11.30$ vs. $30.67 \pm 4.74$ seconds, $t(8) = 4.25$, $p < .01$).
Entries. The amount of entries a rat made into each box was recorded as another measure of how the rat valued the reward associated with each box. A 2x3 factorial ANOVA of the first three-week session found a box by week interaction, $F(2,16) = 3.74, p < .05$. Pairwise t-tests found a trending difference between boxes in W1 (0 pellets < 0/3 pellets: $M = 13.33 \pm 1.04$ vs 16.89 ± 1.24 seconds, $t(8) = -2.27, p = .053$).

There were no main effects of box or week or a box by week interaction for the second three-week session.

Latencies. Latencies refer to the time (in milliseconds) between pellets being dispensed and the rat’s attempt to retrieve them. A lower latency would reflect a higher reward value and possibly impulsivity. A 2x3 factorial ANOVA of the first three-week session showed a box by week interaction, $F(2,16) = 6.06, p < .05$. Pairwise t-tests found a significant difference between boxes in W1 and W2 (W1: 0 pellets < 0/3 pellets: $M = 538.89 \pm 102.48$ vs. 892.00 ± 25.06 milliseconds, $t(8) = -3.56, p < .01$; W2: 1 pellet > 0/3 pellets: $M = 885.07 \pm 28.02$ vs. 749.37 ± 41.38 milliseconds, $t(8) = 2.31, p < .05$).

There were no main effects of box or week or a box by week interaction for the second three-week session.

Food Cup Checks. Food cup checks are believed to be an indicator of the compulsive need to check for reward. A 2x3 factorial ANOVA of the first three-week session revealed a main effect of box, $F(1,8) = 14.18, p < .01$, and a box by week interaction, $F(2,16) = 18.68, p < .01$. Pairwise t-tests found significant differences between boxes in W1 and W3 (W1: 0 pellets < 0/3 pellets: $M = 17.22 \pm 3.51$ vs. 917.11 ± 130.62 checks, $t(8) = -6.83, p < .01$; W3: 2 pellets > 0/3 pellets: $M = 522.78 \pm 94.95$ vs. 223.00 ± 62.79 checks, $t(8) = 2.76, p < .05$).
A 2x3 factorial ANOVA of the second three-week session showed a box by week interaction, $F(2,16) = 12.75, p < .01$. Pairwise t-tests found significant differences between boxes in W6 (1 pellet > 0/1 pellets: $M = 838.89 \pm 134.80$ vs. $101.78 \pm 22.34$, $t(8) = 5.57$ checks, $p < .01$).

_Ultrasonic Vocalizations._ There were no significant main effects or interactions for ultrasonic vocalizations over either three-week session.

_Relative Reward / Choice Discrimination_

Measurements taken from single boxes were compared between weeks to assess the relative reward effect (comparing the same reward magnitude over three weeks) and choice discrimination (comparing the different amounts of reward magnitude over three weeks).

_Total Pellets Consumed._ A one-way ANOVA for total pellets consumed in the single-outcome box for the first three-week session found a week main effect, $F(2,16) = 37.17, p < .01$. Pairwise t-tests found significant differences between all weeks (0 pellets < 1 pellet: $M = 0 \pm 0$ vs. $101.56 \pm 16.47$ pellets, $t(8) = -6.17, p < .01$; 0 pellets < 2 pellets: $M = 0 \pm 0$ vs $191.33 \pm 25.05$ pellets, $t(8) = -7.64, p < .01$; 1 pellet < 2 pellets: $M = 101.56 \pm 16.47$ vs. $191.33 \pm 25.05$ pellets, $t(8) = -3.73, p < .01$; see _Figure 5_). This indicates that the rats were able to discriminate between the advantageous and disadvantageous reward choice based on magnitude and delay.

A one-way ANOVA of the mixed-outcome box for the first three-week session (pellet magnitude is 0/3 pellets for all three weeks) found a week main effect, $F(2,16) = 17.44, p < .01$. Pairwise t-tests found a significant difference between all weeks ($W1 > W2$: $M = 185.5 \pm 21.44$ vs. $98.33 \pm 20.18$ pellets, $t(8) = 3.45, p < .01$; $W1 > W3$: $M = 185.5 \pm 21.44$ vs. $53.39 \pm 15.74$ pellets, $t(8) = 5.44, p < .01$; $W2 > W3$: $M = 98.33 \pm 20.18$ vs. $53.39 \pm 15.74$ pellets, $t(8) = 2.5, p$
< .05). This shows that the relative value of the 0/3 choice decreased as the single-outcome reward increased in absolute magnitude.

A one-way ANOVA of the single-outcome box over the second three-week session (pellet magnitude is one pellet for all three weeks) found a week main effect, $F(2,16) = 15.52, p < .01$. Pairwise t-tests found a significant difference between W4 and W6, as well as W5 and W6 (W4 < W6: $M = 92.0 \pm 13.74$ vs. $186.56 \pm 18.53$ pellets, $t(8) = -7.31, p < .01$; W5 < W6: $M = 106.22 \pm 20.56$ vs. $186.56 \pm 18.53$ pellets, $t(8) = -3.79, p < .01$). This indicates that the relative value of the constant 1-pellet reward increased in W6 relative to W4 and W5.

A one-way ANOVA of the mixed-outcome box over the second three-week session found a week main effect, $F(2,16) = 13.87, p < .01$. Pairwise t-tests revealed a significant difference between W4 and W6, as well as W5 and W6 (0/5 pellets > 0/1 pellets: $M = 134.67 \pm 21.94$ vs. $11.89 \pm 2.61$ pellets, $t(8) = 6.01, p < .01$; 0/3 pellets > 0/1 pellets: $M = 91.61 \pm 21.83$ vs $11.89 \pm 2.61$ pellets, $t(8) = 3.6, p < .01$).

**Total Time in Box.** A one-way ANOVA of the single-outcome box for the first three-week session found a week main effect, $F(2,16) = 18.69, p < .01$. Pairwise t-tests showed a significant difference between W1 and W2, as well as W1 and W3 (0 pellets < 1 pellet: $M = 289.12 \pm 32.71$ vs. $761.47 \pm 91.65$ seconds, $t(8) = -5.45, p < .01$; 0 pellets < 2 pellets: $M = 289.12 \pm 32.71$ vs. $898.84 \pm 108.85$ seconds, $t(8) = -5.45, p < .01$).

A one-way ANOVA of the mixed-outcome box over the first three-week period found a week main effect, $F(2,16) = 19.89, p < .01$. Pairwise t-tests found a significant difference between W1 and W2, as well as W1 and W3 (W1 > W2: $M = 1075.0 \pm 83.62$ vs. $697.08 \pm 99.26$ seconds, $t(8) = 6.52, p < .01$; W1 > W3: $M = 1075.0 \pm 83.62$ vs. $522.09 \pm 89.22$ seconds, $t(8) = 5.63, p < .01$).
A one-way ANOVA of the single-outcome box over the second three-week session found a week main effect, $F(2,16) = 8.19, p < .01$. Pairwise t-tests found significant differences between W4 and W6, as well as W5 and W6 (W4 < W6: $M = 647.59 \pm 73.6$ vs. $1106.75 \pm 84.17$ seconds, $t(8) = -5.25, p < .01$; W5 < W6: $M = 697.84 \pm 120.19$ vs. $1106.75 \pm 84.17$ seconds, $t(8) = -2.61, p < .05$).

A one-way ANOVA of the mixed-outcome box over the second three-week session found a week main effect, $F(1.23, 9.87) = 8.29, p < .05$. Pairwise t-tests revealed a significant difference between W4 and W6, as well as W5 and W6 (0/5 pellets > 0/1 pellet: $M = 760.56 \pm 82.74$ vs. $306.44 \pm 49.13$ seconds, $t(8) 8.29, p < .01$; 0/3 pellets > 0/1 pellet: $M = 676.1 \pm 119.2$ vs. $306.44 \pm 49.13$ seconds, $t(8) = 2.68, p < .05$).

**Box Bouts.** A one-way ANOVA of the single-outcome box over the first three-week session found a week effect, $F(2,16) = 7.78, p < .01$. Pairwise t-tests found significant differences between W1 and W2, as well as W1 and W3 (W1 < W2: $M = 22.17 \pm 2.89$ vs. $62.71 \pm 15.86$ seconds, $t(8) = -2.43, p < .05$; W1 < W3: $M = 22.17 \pm 2.89$ vs. $75.73 \pm 13.25$ seconds, $t(8) = -4.29, p < .01$).

There was no main effect of week for the mixed-outcome box in the first three-week session.

A one-way ANOVA of the single-outcome box over the second three-week session found a main effect of week, $F(2,16) = 6.5, p < .01$. Pairwise t-tests showed significant differences between W4 and W6, as well as W5 and W6 (W4 < W6: $M = 50.24 \pm 6.3$ vs. $81.61 \pm 11.3$ seconds, $t(8) = -2.85, p < .05$; W5 < W6: $M = 47.83 \pm 8.48$ vs. $81.61 \pm 11.3$ seconds, $t(8) = -2.63, p < .05$).
There was no main effect of week for the mixed-outcome box in the second three-week session.

*Entries.* There was no main effect of week for the single-outcome or mixed-outcome box over any of the three-week sessions.

*Latencies.* A one-way ANOVA of the single-outcome box for the first three-week session revealed a main effect of week, $F(2,16) = 6.69, p < .01$. Pairwise t-tests found a significant difference between W1 and W2 (0 pellets < 1 pellet: $M = 538.89 \pm 102.48$ vs. $885.07 \pm 28.02$ entries, $t(8) = -3.59, p < .01$).

There was no main effect of week for any of the other three-week sessions.

*Food Cup Checks.* A one-way ANOVA of the single-outcome box for the first three-week session found main effect of week, $F(1.138, 9.106) = 16.28, p < .01$. Pairwise t-tests found significant differences between W1 and W2, as well as W1 and W3 (0 pellets < 1 pellet: $M = 17.22 \pm 3.51$ vs. $319.33 \pm 36.87$ checks, $t(8) = -8.0, p < .01$; 0 pellets < 2 pellets: $M = 17.22 \pm 3.51$ vs. $522.78 \pm 94.95$ checks, $t(8) = -5.26, p < .01$).

A one-way ANOVA of the mixed-outcome box for the first three-week session revealed a main effect of week, $F(2,16) = 15.72, p < .01$. Pairwise t-tests found significant differences between W1 and W2, as well as W1 and W3 (W1 > W2: $M = 917.11 \pm 130.62$ vs. $497.11 \pm 129.47$ checks, $t(8) = 3.73, p < .01$; W1 > W3: $M = 917.11 \pm 130.62$ vs. $223.0 \pm 62.79$ checks, $t(8) = 4.99, p < .01$).

A one-way ANOVA of the single-outcome box over the second three-week session found a main effect of week, $F(2,16) = 11.91, p < .01$. Pairwise t-tests found significant differences between W4 and W5, as well as W5 and W6 (W4 < W6: $M = 346.78 \pm 67.11$ vs $838.89 \pm 134.8$
checks, $t(8) = -5.04$, $p < .01$; W5 $< W6$: $M = 400.56 \pm 87.58$ vs. $838.89 \pm 134.8$ checks, $t(8) = -3.09$, $p < .05$).

A one-way ANOVA of the mixed-outcome box over the second three-week period found a main effect of week, $F(2,16) = 7.52$, $p < .01$. Pairwise t-tests found significant differences between W4 and W6, as well as W5 and W6 (0/5 pellets $> 0/1$ pellet: $M = 470.89 \pm 82.35$ vs. $101.78 \pm 22.34$ checks, $t(8) = 4.69$, $p < .01$; 0/3 pellets $> 0/1$ pellet: $M = 466.0 \pm 138.34$ vs. $101.78 \pm 22.34$ checks, $t(8) = 2.71$, $p < .05$).

**Ultrasonic Vocalizations.** There was no main effect of week for the single-outcome or mixed-outcome box over any of the three-week sessions.

**Choice Behavior During the First Ten Minutes of Testing**

*Trials.* We ran analyses on data taken from the first ten minutes of the testing session to see if patterns were similar to those of the entire thirty-minute session. A difference between the first ten minutes of each session may reflect that some groups are showing a higher level of motivation to obtain reward than others. The animals may be trying to gain the maximum amount of reward possible at the beginning of the session as opposed to others spacing out their preference over the entire thirty minutes.

A 2x3 factorial ANOVA of the first three-week session found no main effects or interactions.

A 2x3 factorial ANOVA of the second three-week session found a main effect of box, $F(1,8) = 19.27$, $p < .01$, a main effect of week, $F(2,16) = 17.06$, $p < .01$, and a box by week interaction, $F(2,16) = 16.9$, $p < .01$. Pairwise t-tests revealed significant differences between boxes in W6 (1 pellet $> 0/1$ pellet: $M = 59.22 \pm 4.34$ vs. $10.67 \pm 2.06$ trials, $t(8) = 8.47$, $p < .01$).
Food Cup Checks. A 2x3 factorial ANOVA of the first three-week session found a box main effect, \( F(1, 8) = 9.95, p < .05 \), as well as a box by week interaction, \( F(2,16) = 10.9, p < .01 \). Pairwise t-tests found significant differences between boxes in W1 and W3 (W1: 0 pellets < 0/3 pellets: \( M = 15 \pm 4.1 \) vs. 289.44 ± 66.58 checks, \( t(8) = -3.98, p < .01 \); W3: 2 pellets > 0/3 pellets: \( M = 172.89 \pm 35.37 \) vs. 85.11 ± 20.69 checks, \( t(8) = 2.43, p < .05 \)).

A 2x3 factorial ANOVA of the second three-week period found a box by week interaction, \( F(2,16) = 15.83, p < .01 \). Pairwise t-tests found significant differences between boxes in W4 and W6 (W4: 1 pellet < 0/5 pellets: \( M = 91.56 \pm 22.01 \) vs. 212.67 ± 38.62 checks, \( t(8) = -2.35, p < .05 \); W6: 1 pellet > 0/1 pellet: \( M = 267.56 \pm 38.33 \) vs. 49.11 ± 14.4 checks, \( t(8) = 6.35, p < .01 \)).

Relative Reward Effect /Choice Discrimination During the First Ten Minutes of Testing Trials. A one-way ANOVA of the single-outcome box over the first three-week session found a main effect of week, \( F(2,16) = 10.09, p < .01 \). Pairwise t-tests found significant differences between W1 and W2, as well as W1 and W3 (0 pellets < 1 pellet: \( M = 7.33\pm 2.42 \) vs. 32.0 ± 4.47 trials, \( t(8) = -4.68, p < .01 \); 0 pellets < 2 pellets: \( M = 7.33\pm 2.42 \) vs. 32.0 ± 5.31 trials, \( t(8) = -3.82, p < .01 \)).

There was no main effect for week for the mixed-outcome box over the first three-week session.

A one-way ANOVA of the single-outcome box over the second three-week session found a main effect of week, \( F(2,16) = 19.61, p < .01 \). Pairwise t-tests found significant differences between W4 and W6, as well as W5 and W6 (W4 < W6: \( M = 27.67 \pm 4.52 \) vs. 59.22 ± 4.34 trials, \( t(8) = -4.95, p < .01 \); W5 < W6: \( M = 34.67 \pm 4.28 \) vs. 59.22 ± 4.34 trials, \( t(8) = -4.32, p < .01 \)).
A one-way ANOVA of the mixed-outcome box over the second three-week session found a main effect of week, $F(1.24, 9.92) = 8.76, p < .05$. Pairwise t-tests found significant differences between W4 and W6, as well as W5 and W6 (0/5 pellets > 0/1 pellets: $M = 21.89 \pm 2.51$ vs. $10.67 \pm 2.06$ trials, $t(8) = 4.73, p < .01$; 0/3 pellets > 0/1 pellets: $M = 21.44 \pm 4.0$ vs. $10.67 \pm 2.06$ trials, $t(8) = 2.66, p < .05$).

*Food Cup Checks.* A one-way ANOVA of the single-outcome box for the first three-week session found a main effect of week, $F(1.21, 9.71) = 11.42$. Pairwise t-tests revealed significant differences between W1 and W2, as well as W1 and W3 (0 pellets < 1 pellet: $M = 15.0 \pm 4.1$ vs. $80.44 \pm 16.96$ checks, $t(8) = -4.18, p < .01$; 0 pellets < 2 pellets: $M = 15.0 \pm 4.1$ vs. $172.89 \pm 35.37$ checks, $t(8) = -4.33, p < .01$).

A one-way ANOVA of the mixed-outcome box for the first three-week period found a main effect of week, $F(2,16) = 7.49, p < .01$. Pairwise t-tests found significant differences between W1 and W3, as well as W2 and W3 (W1 > W3: $M = 289.44 \pm 66.58$ vs. $85.11 \pm 20.69$ checks, $t(8) = 3.22, p < .05$; W2 > W3: $M = 183.33 \pm 44.1$ vs. $85.11 \pm 20.69$ checks, $t(8) = 2.36, p < .05$).

A one-way ANOVA of the single-outcome box over the second three-week session found a main effect of week, $F(2,16) = 12.72, p < .01$. Pairwise t-tests found significant differences between W4 and W6, as well as W5 and W6 (W4 < W6: $M = 91.56 \pm 22.0$ vs. $267.56 \pm 38.33$ checks, $t(8) = -4.12, p < .01$; W5 < W6: $M = 137.56 \pm 27.11$ vs. $267.56 \pm 38.33$ checks, $t(8) = -3.23, p < .05$).

A one-way ANOVA of the mixed-outcome box over the second three-week session found a main effect of week, $F(2,16) = 14.08, p < .01$. Pairwise t-tests found significant differences between all weeks (0/5 pellets > 0/3 pellets: $M = 212.67 \pm 38.62$ vs. $160.44 \pm 41.23$
checks, \( t(8) = 2.54, p < .05 \); 0/5 pellets > 0/1 pellets: \( M = 212.67 \pm 38.62 \) vs. 49.11 ± 14.4 checks, \( t(8) = 4.85, p < .01 \); 0/3 pellets > 0/1 pellets: \( M = 160.44 \pm 41.23 \) vs. 49.11 ± 14.4 checks, \( t(8) = 2.96, p < .05 \).

**Between Group Analyses**

**Training**

**Trials.** There were no significant lesion effects during training. This illustrates that all animals display preference during the forced choice trials in the same way as control animals.

**Choice Behavior During Open Testing**

**Latencies.** A 3x2x3 mixed ANOVA found a significant box by lesion interaction for choice behavior, \( F(2,25) = 3.51, p < .05 \). From looking at Figure 11 we can see that when taking into account only lesion group and box over the first three-week session, there is no significant difference between lesion groups in the mean latency to retrieve pellets. In the mixed-outcome box, there was a significant difference between the sham lesion group and the dorsal lesion group (\( M = 818.61 \pm 35.37 \) vs. 667.86 ± 32.0 milliseconds, \( p < .01 \)). This shows that the dorsal lesion group was retrieving pellets faster in the mixed-outcome box over the first three-week period than the sham lesion group.

**Measures with non-significant findings.** There were no lesion main effects or interactions for any of the following dependent measures: total pellets consumed, total time in box, box bouts, entries, food cup checks, and ultrasonic vocalizations.

**Relative Reward / Choice Discrimination**
**Total Pellets Consumed.** A 3x3 mixed ANOVA found a main effect of lesion for the relative reward effect in the second three-week session (1 pellet delivery), $F(2,27) = 3.95, p < .04$. LSD comparisons found that the ventral lesion group consumed significantly more pellets in the single-outcome box over this three-week period than the dorsal lesion ($M = 160.7 \pm 11.1$ vs. $119.2 \pm 10.6$ pellets, $p < .02$) group. There was a near significant difference between the ventral lesion group and the sham lesion group over this time period ($M = 160.7 \pm 11.1$ vs. $128.26 \pm 11.7$ pellets, $p = .054$). There were no significant interactions.

**Latencies.** A 3x3 mixed ANOVA found a significant week by lesion interaction in the single-outcome box over the first three-week session, $F(4,52) = 3.16, p < .03$. Post-hoc analyses revealed that the sham lesion group had a significantly lower latency to retrieve pellets in the single-outcome box in week 1 than the dorsal group ($M = 538.89 \pm 78.25$ vs. $793.6 \pm 70.79$ milliseconds, $p < .03$) as well as the ventral lesion group ($M = 849.55 \pm 74.24$ milliseconds, $p < .01$). This indicates that the sham lesion group was responding faster to pellets being dispensed in the single-outcome box over first three weeks than the other two groups. There were no other significant differences between groups over the first three-week session in the single-outcome box.

A 3x3 mixed ANOVA of the first three-week session in the mixed-outcome box found a significant main effect of lesion, $F(2,26) = 5.10, p < .02$. LSD multiple comparisons found that the sham lesion group was retrieving pellets slower than the dorsal lesion group in the mixed-outcome box over the second three-week session ($M = 818.61 \pm 35.37$ vs. $667.86 \pm 32.0$ milliseconds, $p < .01$). There were no other significant differences between groups.
Measures with non-significant findings. There were no lesion main effects or interactions for any of the following dependent measures: total time in box, box bouts, entries, food cup checks, and ultrasonic vocalizations.

Choice Behavior During the First Ten Minutes of Testing

Trials. A 3x2x3 mixed ANOVA found a significant main effect of lesion, $F(2,25) = 3.38, p = .05$. LSD multiple comparisons revealed that the sham lesion group performed significantly less trials in the first ten minutes of testing than the ventral lesion group did when looking at both boxes over all three weeks ($M = 29.26 \pm 1.03$ vs. $33.02 \pm 1.03$ trials, $p < .02$). This tells us that over the first three weeks of testing, regardless of which box and week we are observing, the group that received ventral striatal lesions was performing more trials in the first ten minutes of the session. There were no other significant interactions.

Food Cup Checks. A 3x2x3 mixed ANOVA found a significant week by lesion interaction for food cup checks over the first three weeks of testing, $F(4,50) = 2.63, p < .05$. Post-hoc analyses failed to detect any significant differences between groups. Based on visual inspection of Figure 13, we can see that the sham lesion group remains relatively stable in the amount of food cup checks they perform over the first three week period, while the dorsal lesion and ventral lesion groups increase from week 1 to week 2. The ventral group remains stable for the next week, while the dorsal lesion group drops back down about to where they were in week 1.

Relative Reward / Choice Discrimination During the First Ten Minutes of Testing
There were no significant lesion effects or interactions for trials or food cup checks within the first ten minutes of testing when looking at the relative reward effect and choice discrimination.
CHAPTER V: DISCUSSION

Choice Behavior: A New Paradigm

Our 3-box setup is a new paradigm that can be used to evaluate how rats compare one choice to another. In this study, rats had to choose between reward magnitudes that varied over the weeks, while the delay to deliver the reward remained the same. The rats were given two training days every week to learn which choice was more advantageous when considering both magnitude and delay. Since there is a slight delay before delivery in either box, we considered the box that has the greatest overall magnitude to be the advantageous choice, although in the mixed-outcome box, there could appear to be a total delay of 15 seconds between deliveries of two “0” pellet options.

For most measures, the choice behavior of the sham animals in the first three-week period fit the proposed star model, although there were points where their behavior varied from the model. In terms of total pellets consumed, the sham-lesioned rats showed that they preferred the 0/3 pellet reward as opposed to the 0 pellet reward in week 1 of testing. This preference disappeared in week 2 when they began to prefer the 1 pellet reward equally to the 0/3 pellet reward. In week 3, we see them shift their preference, this time to the 2 pellet reward being more valued than the 0/3 pellet reward. The second three-week period did not yield results that fit the star model as well. Rats did not show a clear preference for the 0/5 pellet over the 1-pellet reward, nor did they show a clear preference for the 0/3 pellet reward over the 1-pellet reward. The only clear preference came in week 6 when rats showed more of a preference to the 1-pellet reward over the 0/1 pellet reward. The lack of a preference for the 0/5 and 0/3 rewards may not be due to magnitude not being large enough, rather it could reflect an aversion to the possible longer delay that occurs when receiving 0 pellets. We did not believe the varying delay would
have this large of an influence when creating the star model. This delay aversion can be attributed to delay discounting, which is where a reward loses its value as the delay to receive it increases (Odum, 2011). It has been shown that humans and non-human animals apply delay discounting differently. Humans discount rewards with higher values less steeply than those with lower values (Green, Myerson, & McFadden, 1997). This is referred to as a magnitude effect. Evidence for rats discounting based off of a magnitude effect has not been as clear as the human evidence. Calvert, Green, and Meyerson (2010) tested this with quality of reward as well as magnitude, and they found that there was no difference for either factor. They claim that this means rats are discounting mainly on the length of delay. Varying the amount of delay in each box as opposed to magnitude could lead the rats to make choices that align more closely with our star model.

To develop the star model, we took into account the idea of scaling. Scaling states that incentive values can change between rewards, but behavior will remain the same if the ratio of change between rewards is the same, even though the magnitude is different (Pellegrini & Papini, 2007). For example, Pellegrini and Papini (2006) showed that rats show similar consummatory behavior when rewards were downshifted, whether they were downshifted from a 32 – 4 % sucrose solution or a 16 – 2 % sucrose solution. Even though the absolute concentrations differed, the ratio of change between rewards was the same, thus leading to similar behaviors in the rats. In our study, the reward magnitude changes between weeks, but the change in ratio remains the same. One difference is that instead of a downward contrast, we introduce an upward contrast (W1: 0 pellets, W2: 1 pellet, W3: 2 pellets). Some have argued that it may be difficult to see this same effect with upward contrast because of a ceiling effect in rat performance (Bower, 1961; Flaherty, 1996). This did not appear to be the case in the present
study as there was a similar difference of total pellets consumed (W1: 0 pellets, W2: 101.56 ± pellets, W3: 191.33 ± 25.05 pellets) between weeks in the single-outcome box when the reward magnitude changed by the same ratio.

By measuring choices between weeks, we can see that the sham-lesion rats were able to discriminate between the single-outcome reward magnitudes in the first three-week period, and they were also able to discriminate between the mixed reward magnitudes in the second three-week period. When looking specifically at total pellets consumed in the single-outcome box, we see a significant increase in the amount of reward obtained when upshifting from the 0-pellet reward in week 1 to the 1-pellet reward in week 2 and again from the 1-pellet reward in week 2 to the 2-pellet reward in week 3. We see a similar pattern in the mixed-outcome box from weeks 4 through 6, although the gap at week 5 is not significant. There are two possible ways to interpret this data. First, we could conclude that the rats were successfully discriminating between changing pellet amounts between weeks. In other words, the rats were correctly comparing 0 pellets to 1 pellet, and so on. These results may also suggest that the rats were only comparing the rewards they were currently experiencing, and the value of each reward was reflected only by its comparison to the reward in the other box. By looking between weeks, we have to assume that the rats were comparing their current reward to a memory of the reward in that same box from the previous week. This 7-day time span is longer than what is typically observed between reward comparisons. Previous studies (Crespi, 1942; Pellegrini & Papini, 2007) have typically used shorter periods, such as 40 minutes to 24 hours, between reward comparisons to enable the rats to make more of a sensory comparison (what they are directly experiencing) than a memory comparison (a reflection of what they experienced before). Having
more time between testing blocks permits more opportunity for interference to occur in that memory.

Another component of reward comparison that the 3-box setup allows us to observe was drawn from comparing reward magnitudes in one box that remained constant over the 3-week time periods. This is known as the relative reward effect. The relative reward effect states that the subjective values of these rewards should change as the animal experiences them and compares them to other experienced rewards, while the absolute value of the reward remains the same. Crespi (1942) stated that in order for the animal to assign a subjective value to a reward, it must first actively experience the reward. By giving the animals two days of training in the 3-box setup, we allow sufficient time for them to actively engage with the reward and assign it a value without confounding this experience with overtraining (Adams, 1982). The relative reward effect was measured in the mixed-outcome box over the first-three week period and in the single-outcome box over the second three-week period. Although the absolute value of the 0/3 pellets in the mixed-outcome box remained the same over the initial three-week period, its subjective value significantly decreased from week 1 to week 2 and again from week 2 to week 3. This can be attributed to the increase in absolute, as well as subjective, value of reward in the single-outcome box over this time span. The opposite occurs for the 1-pellet reward from week 4 to week 6. Its subjective value is significantly higher in weeks 4 and 5 when compared to week 6. Once again, this can be attributed to the 1 pellet reward being compared to the changing amount of pellets in the opposing box over the time span. When rats show behaviors that reflect an increase in subjective value such as that in the single-outcome box in the first three weeks, they are showing what is known as positive contrast (Flaherty, 1996). When behaviors reflect a decrease in value, this is referred to as negative contrast. Crespi (1942) referred to the behaviors as “elation” and
“depression”, but these terms were not as accepted due to the attachment of emotional processes to the words (Flaherty, 1996).

In order to examine if choice behavior in the first ten minutes of testing was similar to choice behavior over the entire testing period, we investigated how many food cup checks each rat made in the first ten minutes. Due to limitations in software, only trials and food cup checks were able to be broken down into ten-minute intervals. Differences between the observed choice behavior in the first ten minutes and the entire session could reflect a difference in how the animal is evaluating the reward at the time of testing. For example, one difference between these times comes in the mixed-outcome box in weeks 4 and 5. In the first ten minutes of testing, rats were checking the food cup significantly more for the 0/5 reward than the 0/3 reward. This difference disappears when we look at the entire session. We see a similar effect when looking at choice behavior between boxes in week 4 (1 pellet versus 0/5 pellets). In the first ten minutes of testing, rats were checking for the 0/5-pellet reward significantly more than the 1-pellet reward, but this also disappears when looking at the entire 30-minute session. It is possible that these discrepancies could occur because the rats are updating their values of the rewards as the session continues. Their initial reactions may be a result of impulsively seeking a reward or an increase in motivation due to food deprivation.

From our results of the sham-lesion rats, there are two conclusions about the 3-box paradigm that we can draw. First, we can say that the rats were able to make what we predicted to be the more advantageous choice when taking both reward magnitude and delay into account. Also, the 3-box setup can be a useful and viable tool for measuring reward choice and decision making. Given these two findings, we are able to accurately draw out any effects that lesions may have on choice behavior. These effects will be reflected upon in the next section.
Although there are many advantages to our new paradigm, there are also some limitations that come with the exploration of a novel paradigm. In both three-week periods, we scaled the difference between the changing pellet amounts to be equal in ratio (Pellegrini & Papini, 2007). Had we used magnitudes that were not different in size ratio, perhaps we could have elicited different behaviors in the rats in the second three-week session that would have fit the star model more closely. Also, given that this paradigm has not been used before, we were unable to come up with a more specific algorithm that could provide us with a better picture of what would be considered optimal or advantageous decision making. For example, Logan (1965) attempted to determine a point of indifference. This is the point at which a delay is long enough to offset a specific magnitude difference. Logan suggested one approach to modeling was to assign a number to a relative value of the reward, regardless if it was a delay or magnitude that was getting the number assigned to it. Larger numbers would indicate that the reward would have a stronger preference than numbers with lower values. If the rewards were equally preferred, the number would be the same. For example, you would assign the same number to a 1-pellet reward that was delivered after 1 second as a 3-pellet reward delivered after 10 seconds if these two rewards were equally preferred. One issue with Logan’s model that he states is that there are still individual aspects choice preference that are unable to be isolated, thus his model may only give a “crude estimate” of choice preference (Logan, 1965). Other, more advanced algorithms have been created to estimate the probability that rats employ a strategy of reward choice that is advantageous (Skelin et al., 2014). An order effect also plays a role in determining which choices the rats may make, but for this study, adding all possible combinations of week order was not feasible. Further use of this setup may provide us with more information to be able to accurately construct a clearer model of reward choice.
Conducting more studies with the 3-box setup would also allow us to investigate some measures that are common amongst other reward choice paradigms. For instance, we could adjust the probabilities of reward delivery in each box, as probability of delivery is one method often used to examine reward choice (Skelin, et al., 2014). Another measure that would be interesting to employ in the 3-box setup would be the effects of hormone levels on reward choice. Uban and colleagues (2012) have shown that estradiol is a key modulator in determining reward choice when using female rats. We could examine this further, or possibly look into how other hormones may affect reward choice using the 3-box setup.

To reiterate, the 3-box setup has proven to be a viable model to measure reward choice and decision making. This novel paradigm provides experimenters with the opportunity to measure many dependent variables. Another large advantage that comes with this setup is that it combines many of the features of other reward paradigms. It provides the linearity and distance of a runway track, the discrimination of a t-maze, the options of a free-choice experiment, the environment of a conditioned place preference task, and the opportunity to include operant tasks. It also provides a novel way to study the effects of lesions to certain brain regions.

**Effects of Striatal Lesions**

As mentioned, the 3-box setup is a novel paradigm that has many advantages. One of these advantages is that it requires a very limited amount of training. Adams (1982) has shown that extended periods of training can lead to animals acting more out of habit than based on the value of the reward they gain. Despite these findings, there are still many studies that include overtraining as part of the methodology (Craft et al., 2011; St. Onge et al., 2010). For example, St. Onge and colleagues (2010) trained animals in 4-block sessions that lasted a total of 48 minutes for three to five days. Their rats had to learn to associate different levers with changing
reward delivery probabilities. One group of rats received an extra three to eleven days of training. When reporting their results, they claimed that this group may have differed from the others because of their inability to update probabilities that were not sequential, but given the findings of Adams (1982), their rats may have stopped responding to the reward itself, and they may have been pressing levers out of habit. A benefit to our paradigm is that the only pre-exposure required for the 3-box setup is two twenty-minute sessions to begin each week. Our results show that there were no significant differences between groups by Training Day 2 of each week. These findings indicate that the three groups were learning at the same rate. This could be attributed to the fact that each rat only has to learn which reward magnitude is associated with either box over the course of the week, as opposed to learning how to perform an operant task (Cardinal & Cheung, 2005). Cardinal and Cheung (2005) showed that rats with nucleus accumbens lesions show a significant impairment in the acquisition of an operant task. Our study eliminated any need for the animal to learn to perform an operant task to receive a reward. This shows that the 3-box setup has an obvious advantage of being able to control for any confounds that may occur from different rates of learning caused by lesions to specific brain regions, which would allow us to look more closely at behavioral functions without this possible confound. One of these behavioral functions is impulsivity.

Impulsivity is defined as the inability to wait for a larger, more advantageous option when a smaller, more immediate option is available (Cardinal et al., 2001). There were two findings in this experiment that reflect the impulsive nature of animals that receive lesions to the ventral striatum. First, when observing the second three-week period, we see the ventral striatal group consuming significantly more pellets in the box that is only dispensing 1 pellet at a time when compared to the group with lesions of the DS (ventral compared to sham was nearly
significant, \( p = .054 \). Week 4 consists of the box that has the highest absolute pellet total (2.5 pellets per trial) of all six weeks, while the single-outcome box only dispenses 1 pellet per trial. While performing more trials in the single-outcome box during week 6 is advantageous, this performance by the VS group is not considered optimal decision making during the other two weeks of this testing period. The other finding representing the impulsive nature of this group was that the VS group performed significantly more trials than the sham lesion group in the first ten minutes of testing over the first three-week period, regardless of box. These findings fall in line with those of previous studies (Cardinal et al., 2001). The increase in impulsive behavior in animals with lesions to this brain region has led to the belief that the VS (specifically the nucleus accumbens) is a key structure in disorders such as drug addiction (Nestler, 2013) and pathological gambling (Reuter et al, 2005). While the VS is the main structure involved in these impulse-control disorders, the DS plays a key role in other disorders, some of which also include decision making.

The DS is a key component of the nigrostriatal system (Horvitz, 2000). This system plays a large role in motor control (Chagniel et al., 2012) and dysfunction of this system is mostly associated with Parkinson’s disease and Huntington’s Disease (Nelson & Kreitzer, 2014). Given this, we could expect to see motor impairments in the group of rats that received DS lesions. Other studies have supported this idea (Gengler, Mallot, & Hölscher, 2005), although these impairments do not always occur to the same extent (Chagniel et al., 2012). The group of rats that received lesions to the DS in the current study did not show motor impairments. This conclusion can be drawn from the lack of between group significance between entries, as well as the lack of between group differences for most instances that latencies were recorded. There was one instance in the second three-week period, however, where the DS lesion group was actually
retrieving pellets faster than the sham lesion group. This tells us that the dorsal striatal lesions did not lead to any of the typical motor deficits that they potentially can cause. An explanation for why this group would be retrieving pellets faster is still unclear. One possible explanation is that our motor task was very simple in that the rat could hang onto the lip of the food cup and poke its head in to retrieve the pellet. Another explanation would include that the response needed to obtain a pellet in our study is too predictable and not complex in its nature, such as pressing a lever or pulling a chain. Motor deficits are some of the behavioral characteristics that link the DS to Parkinson’s disease, but these are not the only deficits involved with this disease. It has also been shown that people with Parkinson’s disease often have difficulty in tasks involving strategic decision making (Riba, et al., 2008). It is believed that they choose riskier options because there is a decrease in response of the reward system of their brain, therefore, they are trying to overcompensate for this loss of activation. The rats in the current experiment did not show this type of behavior.

Although there are many measures involved in the 3-box setup, there was not an overabundance of significant between group differences in this particular experiment. As it has been eluded to earlier in this paper, it is believed that this can be attributed to the learning curve, or lack thereof, for this design. When doing lesion studies, most other experiments involve some sort of operant behavior (Avila & Lin, 2014; Cardinal & Cheung, 2005). For example, Cardinal and Cheung (2005) found significant differences between groups on a lever pressing task, but these differences began to disappear after an extended amount of sessions. One way the current experiment could be modified in order to be compared with other work is if we were to introduce an operant behavior, such as lever pressing, in order for the rats to gain the reward associated
with each box. Something such as this could possibly draw apart larger group differences and aid even further in understanding how these brain regions function in reward choice.

While we can learn a lot from lesion studies, there are some drawbacks. When we lesion an area of the brain, we are not only taking away that area, but we are also disconnecting any areas that may have connections running through it (Simmons, Ackerman, & Gallistel, 1998). This could mean that we are not only taking away that area of the brain, but corrupting communication between other areas as well. There are a few methods we could use in the future to better understand the VS and DS’s role in decision making and reward choice using the 3-box setup. One of the more simple methods we could use to investigate the role of these brain areas would be to use agonists or antagonists of neurotransmitters that are highly prevalent in these areas. St. Onge and Floresco (2009) have shown that amphetamine effectively works as a dopamine agonist in choice studies, while there are many chemicals, such as eticlopride in their study, that will work as an antagonist. This is one way to effectively keep that area of the brain from working, without completely eliminating it. Another method to examine would be single-unit recordings. Cromwell and Schultz (2003) have shown that there are specific neurons activated in the VS during reward expectancy. This method would allow us to more precisely examine how these brain areas are functioning during the task. Finally, we could use microdialysis to measure the fluctuating amounts of neurotransmitters that are being released during a given task. For example, Ikeda and colleagues (2013) found that activations of neurons in the nucleus accumbens shell have an effect on the amount of dopamine released in the DS. By using some of these alternative methods, we may be able to shed more light on how these brain areas function in reward choice and decision making.
Ultrasonic Vocalizations

USVs have been shown to be an indicator of affect in rats (Knutson, Burgdorf, & Panksepp, 2002). The current study measured the amount of 50 kilohertz USVs emitted as a state of positive affect. While we did find that the sham-lesion rats were emitting USVs, there were no significant differences between weeks. From Figure 14, we can see that USVs emitted by the sham group were relatively stable across all six weeks, with a slight increase for the 1-pellet reward in week 4. The lack of significant findings may come from the relatively small number of subjects that we were able to analyze due to the technological difficulties reported earlier.

We also expected to see a difference of USVs being emitted at the between group level, but there were no significant differences detected. Once again, this may be from the sample size, but it also could be from the large variability that we found. From visual inspection of Figure 14, we can see that there is a pattern of the VS-lesion group having a large spike in USVs emitted from week 4 through week 6, but there is also large variability. The DS-lesion group follows a very similar pattern to that of our sham-lesion group.

The finding that striatal lesions did not result in any between group differences in 50 kilohertz USVs may seem counterintuitive. Since it is believed that the VS and DS play a role in reward evaluation, it would make sense that eliminating these areas would have an effect on the subjective feeling of reward. This effect could possibly be conveyed through changes in 50 kHz USVs, which have been shown to be an indicator of positive affect (Knutson, Burgdorf, & Panksepp, 2002). Previous studies have shown this exact finding. Burgdorf and colleagues (2007) found that VTA lesions or blockage of dopamine receptors in this same area, were able to significantly reduce the amount of 50 kHz USVs. This effect occurred in the same brain areas that they stimulated earlier in the same study and found an increase in 50 kHz USVs.
There are multiple possible explanations for these findings. First, we did not lesion the same brain area as the aforementioned study. The VTA sends dopaminergic input to the VS (Ikemoto & Panksepp, 1999), therefore, we may have disrupted the process at a different stage. In other words, we may not have eliminated the subjective feeling being produced by leaving the VTA intact. By doing this, the animals may still feel pleasure without being able to integrate it with the decision-making occurring in the rest of the mesocorticolimbic system.

Another explanation is based off of the idea of the emotional processes (for a comprehensive overview of emotional processes, see Panksepp & Biven, 2012). These processes can be broken down into three levels. The first level is the primary processes, which are the basic and most primitive emotions. The next level is the secondary level, which integrates learning through the basal ganglia. The final level is the tertiary level, which is our cognitive “awareness” of emotions (Panksepp & Biven, 2012). When we lesion the VS, we are eliminating an important structure within the secondary level of emotions. This level provides much inhibition to the primary level, and it is possible that by eliminating a key component of the secondary level, this is disinhibiting the primary process level of emotion. This could be another explanation for the persistence of USVs in the presence of VS lesions.

Finally, we may not have had a task that would evoke a strong enough emotional response to elicit a large difference in USVs. Our paradigm dispensed a simple sucrose reward, and there was no real penalty involved for poor performance. Also, our animals were only moderately food-deprived (87 - 90% of baseline weight). Other studies investigating USVs use very strong reinforcers such as amphetamine (Wright, Gourdon, & Clarke, 2010) or electrical brain stimulation (Burgdorf et al., 2007) which could lead to a heightened affective state. Burgdorf and colleagues (2000) showed that electrical brain stimulation is a powerful enough
reinforcer to elicit USVs in mere anticipation of the reward. This leads us to believe that we could possibly get a stronger response if we were to induce a more affective experience for the animals.

**Neural Circuitry of Reward Choice**

The VS is a key component to the mesocorticolimbic circuit, which is often referred to as the brain’s reward circuit (Utter & Basso, 2008), although there is some debate as to whether or not it should be labeled the “seeking” circuit due to its ability to promote seeking out reward in comparison to modulating the feelings of reward (Panksepp & Biven, 2012). This circuit contains cell bodies that originate in the VTA and send dopaminergic input to the VS, which then innervates areas of the prefrontal cortex (Ikemoto & Panksepp, 1999).

One issue with studying this circuit is how to decipher if an animal actually finds something rewarding. Panksepp and Biven (2012) state that if you allow the animal to control whether or not something occurs, such as electrical brain stimulation to a rewarding area of the brain, and the animal consistently commits a behavior to continue this stimulation, then you can conclude that the animal finds that specific stimulus rewarding. This has been the case when looking at this specific rewarding circuit. For example, if given the opportunity, rats will self-administer drugs of abuse to the nucleus accumbens once trained in a standard operant task (Phillips, Robbins, & Everitt, 1994). Not only will stimulation to this circuit invoke reward, but as mentioned earlier, disruptions to areas of this circuit can shift reward choice towards a less favorable option (Cardinal & Cheung, 2005). Assigning a value to and liking a reward is only one aspect of choosing it, the animal still has to be motivated to obtain the reward, and that is where the dorsal subsection of the striatum is involved. Although the DS is functionally and
anatomically different from the VS, they both need the neurotransmitter dopamine for proper functioning.

Dopamine is one of the key neurotransmitters in reward choice and motivated behavior (Berridge & Robinson, 1998; Ikemoto & Panksepp, 1999). Substances such as drugs of abuse are believed to be rewarding because they limit the effects of the dopamine transporter, leaving more dopamine available at the synaptic level (Ikemoto & Panksepp, 1999). It has been shown that dopamine is crucial for learning about a reward as well (Morita & Kato, 2014). When an organism first receives a reward, there is an increase in extracellular dopamine, but if that reward is consistently signaled by a cue, the increase in dopamine will shift to the onset of the cue (Schultz, Dayan, & Montague, 1997). Also, dopaminergic neurons are expected to encode the value of a given reward, and if this value differs from what is expected, these neurons will show different activity, and this activity is known as the reward prediction error (Colombo, 2014; Hollerman & Schultz, 1998). Given this, we can assume that VS or DS lesions cause dopamine signaling to be interrupted, thus reward choice is altered. This could be an explanation for unusual decisions being made by the animal.

There are other brain areas that have been implicated in the process of reward choice and decision making, and these could be areas of particular interest in future studies done in the 3-box setup. The ventral pallidum (VP) is one area of the brain that has been investigated recently for its role in reward (Smith, Tindell, Aldridge, & Berridge, 2009). It has been found that the VP and nucleus accumbens share reciprocal connections that are needed in order to increase the “liking” of a reward, but only the nucleus accumbens is needed to “want” the reward (Smith & Berridge, 2007). While it seems the VP is playing a role to promote obtaining reward, the lateral habenula (LHb) seems to be doing the opposite (Ji & Shepard, 2007). When a stimulus that
predicts a non-reward is shown, neurons in the LHb show an increase in firing, while dopaminergic neurons in the substantia nigra show an increase. These findings are reversed when a reward-predicting stimulus is shown (Matsumoto & Hikosaka, 2007). It has also been found that neurons in the LHb are excited during punishment or during the omission of reward (Matsumoto & Hikosaka, 2009). Ji and Shepard (2007) were able to suppress firing of 97% of the dopaminergic neurons in the substantia nigra and VTA by stimulating the LHb. Taken together, these findings indicate that the LHb helps an animal recognize aversive stimuli by inhibiting the firing of the rewarding, dopaminergic neurons, as well as not firing in response to rewarding stimuli. This pattern of firing has been referred to as a negative reward signal (Matsumoto & Hikosaka, 2007). Examining these brain regions and their interactions with the VS and DS could provide us with a clearer understanding of how animals decide between reward choices.

**Conclusion and Future Work**

Reward choice and decision making are behaviors common in all animals. The ability to efficiently and advantageously perform these behaviors can be vital in certain situations. The 3-box paradigm allows us to evaluate how animals make decisions by using a setup that has not been used before. In our novel paradigm, we found that lesions to the VS and DS can disrupt these processes in a limited fashion. This paradigm can be utilized in future studies using techniques involving microdialysis, single-unit recordings, different qualitative rewards, or varying amounts of delay. A better understanding of how these brain areas function in reward choice and decision making can shed light on clinical cases such as substance abuse, pathological gambling, obsessive compulsive disorder, and depression.
REFERENCES


*Behavioural Brain Research, 18* (2), 274 – 283.


*Studies in History and Philosophy of Biological and Biomedical Sciences, 45*, 57-67.


APPENDIX A. IACUC APPROVAL FORM

Dr. Howard C. Cromwell  
Psychology  
Bowling Green State University  

Re: Annual Renewal of IACUC Protocol 12-012  

Title:  
*Brain basis for reward choice behavior*  

Dear Dr. Cromwell:  

On April 16, 2014 the annual renewal for the above referenced protocol was **approved** after review by the IACUC. This renewal is in effect for one calendar year and expires on April 15, 2015. Please consult with the staff of the Animal Facility about any special needs you might have to continue with this project.  

Comment(s):  

Sincerely,  

[Signature]  

Hillary Snyder, Ph.D.  
IACUC Administrator
Figure 1. The Three Box Setup. The three box setup is depicted above. The black bars above the tunnels represent the location of the guillotine doors. Black dots are the locations of all IR beams. At the beginning of each session, the rat is placed in the middle “decision” box. It will have the choice to enter either Box 1 or Box 2, which will each have an assigned reward value. On training day 1 and training day 2, a guillotine door will lower, trapping the rat in the chosen box for ten minutes. The same will occur in the other box after the original ten minutes has passed. On open day 1 and open day 2, the rat will be free to roam all three boxes for thirty minutes. On the extinction day of every week, the rat will be free to roam all three boxes with no reward being delivered.
Figure 2. Above is a visual representation of the proposed “star” model. The solid, black line represents the predicted choice behavior in week 1 and week 4. The dashed, black line represents the predicted choice behavior in week 2 and week 5. The dotted, black line represents the predicted choice behavior in week 3 and week 6. The numbers next to each line represent the pellet magnitude being delivered for that week. Numbers in italics are for weeks 1 – 3, while bold numbers are for weeks 4 – 6.
Figure 3. Choice behavior over the first three-week period for the sham lesion group only (mean ± S.E.). Total reward is measured in the amount of total pellets consumed in the half-hour period of testing. Choice preference shifts from the mixed-outcome box in week 1, to being indifferent in week 2, to the single-outcome box in week 3. These results closely resemble the proposed star model. The * on week 1 and week 3 indicate a significant difference in choice ($p < .01$).
Figure 4. Choice behavior over the second three-week period for the sham lesion group only (mean ± S.E.). Over the second three-week period, we see that the rats have a difficult time choosing between the single-outcome and mixed-outcome box over the first two weeks, but choice preference strongly shifts to the single-outcome box during the final week of testing. The * over week 6 indicates a significant difference between the single and mixed-outcome box ($p < .01$).
**Figure 5.** The above graph illustrates both the relative reward effect and choice discrimination over the first three-week period for the sham lesion group only (mean ± S.E.). The solid black line shows choice discrimination in weeks 1 – 3. During week 1 (0 pellets) the rats were not able to gain any reward. When the magnitude of pellets shifts up in week 2 (1 pellet), the rats preference for this box also shifts up, and this shift continues into week 3 (2 pellets). The dashed line illustrates the relative reward effect (0/3 pellets for all 3 weeks). Here, we see almost the opposite trend, with the highest choice being in week 1, that choice dropping in week 2, and dropping even more in week 3. This shows that as the absolute magnitude of reward increases in the single-outcome box and the absolute magnitude of reward in the mixed-outcome box remains the same, the subjective value of reward in the mixed box decreases. * \( p < .01; ** \( p < .05. \)
Figure 6. The above graph illustrates both the relative reward effect and choice discrimination over the second three-week period for the sham lesion group only (mean ± S.E.). The solid black line shows the relative reward in weeks 4 – 6. From week 4-6, the constant 1 pellet increases in subjective value. Rats gained significantly more total reward in the single-outcome box in week 6 than both week 4 and week 5. The dashed black line represents choice discrimination. The rats consumed significantly less total pellets in week 6 than both week 4 and week 5. *p < .01.
Figure 7. The above graph illustrates choice discrimination during the first three-week session. There were no significant differences between groups. Error bars represent + / - 1 S.E.
Figure 8. The above graph illustrates the relative reward effect for weeks 1 through 3. There were no significant between group differences. Error bars represent +/- 1 S.E.
Figure 9. The above graph represents a main effect of lesion for the relative reward effect for weeks 4 through 6. Post-hoc analyses revealed that regardless of week or box, the ventral lesion group consumed significantly more pellets than the dorsal lesion group in the single-outcome box over the second three-week period \((p < .05)\). Error bars represent \(+/-1\) S.E.
Figure 10. The above graph illustrates choice discrimination during the second three-week session. There were no significant differences between groups. Error bars represent + / - 1 S.E.
Figure 11. This graph represents a significant box by lesion interaction for latency (mean ± S.E.).

While there is a pattern of the ventral lesion and dorsal lesion groups having a slightly higher latency in the single-outcome box than in the mixed-outcome box, the sham lesion group has a higher latency in the mixed-outcome box. There was a significant difference in latency in the mixed-outcome box between the sham lesion group and the dorsal lesion group. *$p < .01$. Error bars represent $+/-1$ S.E.
Figure 12. The above graph illustrates a week by lesion interaction for choice discrimination over the first three-week period. In week 1, the sham lesion group had a significantly lower latency than the ventral lesion and dorsal lesion groups. As the weeks progress, this significant difference disappeared. *$p < .01$; **$p < .05$. Error bars represent +/− 1 S.E.
Figure 13. The above graph illustrates a week by lesion interaction for average food cup checks during the first ten minutes of testing for the first three-week period. This interaction does not take into account which box the food cup checks are being made in. While post-hoc analyses failed to detect any significant differences, we can observe a pattern of the sham lesion group keeping a relatively stable pattern of food cup checking over the three weeks, while the other two groups change over the weeks. The dorsal lesion group shows an increase from week 1 to week 2, followed by a decrease from week 2 to week 3. The ventral lesion group shows a similar increase from week 1 to week 2, while from week 2 to week 3, they show a similar leveling-off pattern that the sham lesion group demonstrated.
Figure 14. The above graph illustrates the amount of 50 kHz USVs emitted by each group in both boxes over the course of 6 weeks. Although there is a large spike in USVs emitted by the VS-lesion group after week 3, there were no significant differences. Error bars represent +/− 1 S.E.