ROLE OF D4 RECEPTORS OF THE PREFRONTAL CORTEX IN EXECUTIVE FUNCTIONING

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ABSTRACT

The Attentional Set Shifting Task (ASST) is a rodent analogue of the Wisconsin Card Sorting Task (WCST), which measures executive functioning. The ASST tests for reversal of stimulus-response learning and the formation and maintenance of attentional sets. $D_4$ receptor antagonism has been shown to improve performance of visual discrimination in drug naïve rats. The study presented here questioned if a $D_4$-specific antagonist, L-745,870, could have a similar effect on animals even after being treated with repeated doses of cocaine, amphetamine or the $D_2/D_3$ receptor agonist quinpirole. Cocaine-treated rats showed impairments in both reversals and attentional shifts, while amphetamine impaired only reversal stages and quinpirole only impaired attentional shifts. All groups, however, when pretreated with the $D_4$ antagonist L-745,870 improved on previously impaired stages. $D_4$ antagonism also increased the latency to respond in some cases. $D_4$ receptors are involved in N-methyl-D-aspartate (NMDA) receptor functioning and striatal-cortical loops that executive functioning depend on. Also, $D_4$ receptors play a role in cue salience and by blocking these receptors, animals display less attachment to previously rewarded cues. Results of the study can help elucidate this role and have implications for targeting $D_4$ receptors as cognitive enhancers.

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# CHAPTER 1

## GENERAL INTRODUCTION

Behavioral flexibility, also known as executive functioning, is described as the ability to alter behavior based on changes to the environment. This can include such tasks as error correction, decision-making, planning, and sequencing (Robbins, Weinberger, Taylor & Morris, 1996). In human research, attentional set-shifting is measured by the Wisconsin Card Sorting Task (WCST). This task requires the participant to match cards based on rules that have not been specified. The rules are learned implicitly based on the participant being told whether or not his or her match is correct. During the task, the rules are changed without the participant's knowledge, and the participant must respond accordingly. Research with healthy subjects as well as patients with brain tumors and lesions indicates that the WCST requires the use of the frontal cortex (Berman et al., 1995; Mestrović, Palmović, Bojić, Treselj & Nevajda, 2012). For example, patients with a tumor in the left frontal cortex tend to make more errors in this task. Similar conclusions have been reached when schizophrenic patients with reduced prefrontal activity or frontal cortex impairments have been tested (Meyer-Lindenberg, et al., 2002; Pantelis, et al., 2009).

In rats, executive functioning can be tested and measured with a paradigm known as the Attentional Set-Shifting Task (ASST), which is an analogue of the WCST in humans. The system of learning and applying unstated rules is applied to the ASST in terms of digging medium and scent. Developed by Birrell and Brown (2000), the ASST trains rats to search in a flower pot for a food reward based on a specific digging medium or scent in a series of stages that target a specific type of learning. The ASST is designed to have a rat form attentional sets and then shift from one previously determined set to another unknown set. The Mackintosh

model describes the attentional set as the understanding that a multidimensional stimulus, in this case the flower pots presented, has certain aspects that either predict a reward or do not. Due to the presence of a reward, one stimulus becomes more salient to the subject and therefore is chosen more often (Esber and Haselgrove, 2011; Mackintosh, 1975). This is the case for both the intradimensional shift (IDS) and extradimensional shift (EDS). The IDS requires the rat maintain the rules of the attentional set but apply them to novel stimuli in the same dimension (e.g., vanilla scent to pine scent) (McAlonan & Brown, 2003). The EDS is the same except that the attentional shift is a switch between the perceptual dimensions of either odor to digging medium or vice versa (e.g., pine scent to dark colored foam) (McAlonan & Brown, 2003). Reversals also require that the animal maintain an attentional set, but it must also learn a new association between what is predicting the reward, namely the reversal of the previous rule (e.g., switching from jasmine scent to vanilla scent when jasmine was previously rewarded). There is no novel stimuli being presented and the perceptual dimension remains the same. The number of trials needed to reach criterion is used as a measure of the formation of the attentional set (McAlonan & Brown, 2003). The different stages of the ASST measure different aspects of executive functioning and are thought to be mediated by different areas of the prefrontal cortex (PFC).

Based on human and animal research, the prefrontal cortex is vital for behavioral flexibility. Lesions to specific areas of the PFC result in different impairments in the different stages of the ASST which suggest that reversal learning and attentional shifts are mediated by different areas of the PFC. For example, when the medial prefrontal cortex (mPFC) is lesioned in rats, performance on the EDS is impaired while all other aspects of the test, reversal learning and initial rule learning, are intact (Birrell & Brown, 2000). When the orbitofrontal cortex (OFC)

is lesioned, reversal learning is impaired and not attentional shifts (McAlonan & Brown, 2000). In marmosets, lesions to the lateral prefrontal cortex (lPFC) and OFC impaired all stages of the ASST save for the IDS, suggesting that these areas are vital for reversal learning and switching sets between perceptual dimensions (EDS) but not necessary for maintaining an attentional set within the same dimension (IDS) (Dias, Robbins & Roberts, 1996). In rats, however, the comparative areas to a marmoset are different. It is thought that the mPFC is analogous to the lPFC of the marmosets and therefore, lesions to these areas impair attentional shifts (Birrell & Brown, 2000).

Behavioral inflexibility is one of the hallmarks of drug abuse most likely due to the damaging effects of drugs on the prefrontal cortex (Schoenbaum, Roesch & Stalnaker, 2006). For example, cocaine and opioid users have been shown to have increased perseverative responding and a greater error rate in the WCST, including showed greater impairments than controls in the extra-dimensional set of the WCST (Colzato et al., 2009; Ersche et al., 2007; Lyvers & Yakimoff, 2003; Soar, Mason, Potton & Dawkins, 2012; Woicik et al., 2011). Specifically, cocaine users showed a significant correlation between the amount of cocaine used and errors made during the task (Soar et al., 2012). In animal models, Schoenbaum, Saddoris, Ramus, Shaham and Setlow (2004) have shown that rats that had two weeks of 30 mg/kg daily injections of cocaine had significant impairments in an odor reversal task. A similar impairment is seen in rats trained to self-administer cocaine, even after a withdrawal period of up to three months (Calu et al., 2007). In mice, Black et al., (2006) saw that a sensitization dosing regimen of cocaine during adolescence, increased the number of trials required in the EDS and resulted in more errors overall when tested in adulthood (Black et al., 2006).

Amphetamine (AMPH), another stimulant also produces deficits in behavioral flexibility during use and withdrawal. Ornstein et al., (2000) tested AMPH users on the WCST and found that they made significantly more errors than controls. They also had higher drop-out (inability to complete a stage) rates in the EDS. Similar results were seen in a study comparing current AMPH and opiate users to abstinent drug users (Ersche et al., 2006). Current AMPH users showed greater impairments and higher error rates in tasks related to executive functioning and memory when compared to opiate and abstinent users. The authors suggest this is due to AMPH's damaging effects on the PFC and medial temporal lobe (Ersche et al., 2006). The effect of methamphetamine, mAMPH, on chronic users similarly reveals WCST impairment, namely, increases in perseverative errors and fewer completed categories (Henry, Minassian & Perry, 2010).

In rats, Izquierdo et al. (2010) showed a binge regimen of mAMPH injections impaired performance on early reversals in rats. Exposure to mAMPH increased the number of trials needed to reach criterion in the reversals. Despite reversal impairment, there was no impairment in the stages testing attentional set-shifting, suggesting mAMPH induces impairments in behavioral flexibility (Izquierdo et al., 2010). Izquierdo et al. (2010) further suggest that the impairments in reversal learning are caused by an mAMPH-induced dopaminergic dysregulation in the striatum, which in turn decreases OFC activity.

The OFC has a high prevalence of $D_2$ receptors, which are often the target of drugs of abuse. Previous reports have shown a decrease in $D_2$ receptor availability in the frontal cortices due to frequent activation during chronic use of both cocaine and amphetamine (Volkow et al., 1993, Volkow et al., 2003). These findings suggest that $D_2$ receptors are a particular target for stimulants. Quinpirole is a selective $D_2/D_3$ receptor agonist, has been used in various studies

observing drug abuse behaviors because it has similar dopamine receptor targets as cocaine and amphetamine. For example, priming injections of quinpirole can increase drug-seeking behaviors (Self, Barnhart, Lehman & Nestler, 1996), mimic cocaine priming injections to induce relapse (Khroyan, Barrett-Larimore, Rowlett & Spealman, 2000), and can induce a place preference in cocaine-treated rats (Graham, Hoppenot, Hendryx & Self, 2006).

Quinpirole, a $D_2/D_3$ agonist, also has been shown to cause marked impairments in tests of behavioral flexibility, similar to both cocaine and amphetamine. A pretreatment injection of quinpirole (0.3 mg/kg) significantly impaired reversal learning by increasing the number of trials needed to reach criterion and by increasing the number of errors committed during reversal trials (Boulougouris, Castañe & Robbins, 2008), indicating a connection between the $D_2$ receptors and reversal-learning impairments. This finding is supported by Haluk and Floresco (2009); however they were able to find impairments in set-shifts as wells as reversal impairments. The authors suggest that abnormal increases in $D_2$ receptor activation create a general impairment in behavioral flexibility (Haluk & Floresco, 2009).

Izquierdo et al. (2010) and Haluk and Floresco (2009) suggest that dopaminergic dyregulation (decreased availability or overactivity of dopamine) is a possible cause for behavioral inflexibility. Evidence suggests that dopamine is a major modulatory factor in behavioral flexibility based on its prevalence in the systems of the PFC (Grace, Floresco, Goto & Lodge, 2007). For example, patients with schizophrenia and Parkinson's or those with attention deficit hyperactivity disorder have difficulty with set-shifting tasks and also have pathologically dysfunctional dopaminergic systems (Floresco & Magyar, 2006; Owen et al., 1993). In marmosets, lesions designed to deplete dopamine while leaving all other catecholamines intact have shown that depleting dopamine in the frontal lobes impairs acquiring and maintaining

attentional sets (Crofts et al., 2001). It is thought that dopamine acts via "top-down" processes in the frontal cortices, meaning the PFC is necessary for mediating a fronto-cortico-striatal loop to ensure proper executive functioning (Krause et al., 2012). Unsurprisingly, PFC-specific dopamine activity displays a U-shaped effect in performance on the ASST versus dopamine levels. The greatest impairments are seen when there is a lack of dopamine (Mattay et al., 2003; Ragozzino, 2002; Williams and Goldman-Rakic, 1995) and an increase in dopamine (Zahrt, Taylor, Mathew, & Arnsten, 1997), while optimal performance is found in mid-range.

The U-shaped effectiveness of dopamine in the prefrontal cortex may be due to the varied pharmacological action of each dopamine receptor. Floresco, Magyar, Ghods-Sharifi, Vexelman, and Tse (2006) showed that each dopamine receptor subtype had differing effects in the ASST. $D_2$ antagonists caused reliable impairments on the task, whereas $D_2$ agonists had no effect of performance. $D_1$ agonists had a negligible effect on performance while an infusion of a $D_1$-receptor antagonist impaired performance. When the $D_4$ antagonist L-745,870 was infused bilaterally to the PFC, performance on a set-shifting task improved, whereas a $D_4$ agonist impaired performance on the same task (Floresco et al., 2006). Work in primates has shown similar results with a $D_4$-specific antagonist in an object retrieval task, even after repeated exposure to phencyclidine (Jentsch et al., 1999). The $D_4$ receptor possibly had this effect due to its abundance in the PFC. $D_4$ receptors are highly localized to the frontal cortex (dorsolateral frontal, medial prefrontal, and entorhinal cortex), cortical regions surrounding the prefrontal cortex, the amygdala and hippocampus (Oak, Oldenhof & Van Tol, 2000; Tarazi, Kula & Baldessarini, 1997; Wędzony, Chocyk, Mackowiak, Fijal & Czyrak, 2000). These areas are included and vital to the fronto-cortical loop and therefore contribute to executive functioning.

Impairments in behavioral flexibility are a common theme among those who currently or previously used drugs of abuse. Damage caused by drug exposure reveals itself in poor decision making, memory impairments, and inability to use predicted behaviors to change responses and behaviors (Schoenbaum, Roesch & Stalnaker, 2006; Volkow, Fowler & Wang, 2003). $D_4$ receptor antagonism has been seen to improve performance in tasks requiring those very skills. The studies presented aim to evaluate the role of $D_4$ receptors in behavioral flexibility. It is known that $D_4$-receptor agonists impair behavioral flexibility while $D_4$-receptor antagonists improve performance; however, this study presented for the first time, the positive effect of $D_4$ antagonists after chronic exposure to drugs of abuse. Subjects were treated with cocaine, amphetamine or quinpirole for 10 days. Half of the animals were then tested, while the other half was pretreated with the $D_4$ antagonist L-745,870. All subjects were tested on the ASST to determine both the impact of the specific drug on executive functioning and the extent to which $D_4$ receptor activity is able to improve performance.

# CHAPTER 2

## EXPERIMENT 1: METHODS AND RESULTS

Cocaine use impairs learning and memory (Colzato et al., 2009; Ersche et al., 2007; Lyvers & Yakimoff, 2003; Soar, Mason, Potton & Dawkins, 2012; Woicik et al., 2011). Explanations often involve cocaine's effect on dopamine. Cocaine results in increased dopamine presence in the synaptic cleft. This availability of dopamine can subsequently downregulate dopamine receptors. Downregulation of dopamine and dopaminergic dysregulation can impair behavioral flexibility. For this reason, cocaine was administered to rats that would receive the ASST to determine its effect on behavioral flexibility. In addition, a subgroup of the cocaine-treated rats was tested on the $D_4$ antagonist, L-745,870. This was done to examine if $D_4$-receptor blockade could improve or reverse the impairment caused by the drugs. Previous reports have shown that $D_4$-receptor blockade can improve performance in drug naïve rats. The result of interest is whether or not the $D_4$ antagonist would display similar effects on rats that have experienced chronic cocaine exposure.

### Subjects

Forty male Long Evans rats aged 50 days on arrival were obtained from Harlan Labs (Indianapolis, Indiana, USA). The animals were individually housed in plastic bins with wood chip bedding and metal wire tops with food and water freely available until they reached three months of age. After 90 days in residence, they were put on a food-restriction diet until they reached 85% of their free-feeding body weight. During food restriction leading up to training, rats were handled daily for twenty minutes per day. The room in which they were housed was maintained at 23°C and kept on a 12-h light/dark schedule of lights on 8 AM. The care of the animals and procedures conducted were approved by the Institutional Animal Care and Use

Committee at American University, which follows the guidelines recommended by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011).

## Learn to Dig Training Phase

Prior to testing, the animals were trained to dig in flower pots for food rewards. The flower pots were terra cotta pots, 6 x 6 inches (height x width; purchased from The Home Depot). The pots were weighed down with gravel and the gravel was covered with melted wax to provide a stable base. After filling with gravel and wax, the pot allowed for 1-2 inches of area to fill with the various digging media. On day one of training, rats were placed in the testing chamber (66-quart plastic bin) with one weighted flower pot filled with paper. A small pellet of rat chow (Dustless Precision Pellets, purchased from Bio-Serv) was placed on top of the paper for an obvious reward. Once the rat had successfully found 10 rewards, day-one training was complete. Day two of training gave five uncovered rewards and five rewards hidden (covered rewards) under the paper that required the rat to dig to find them. Day three of training required 10 covered rewards. On day four, the final day of training, rats needed to find 10 covered rewards each presented on a trial basis with an inter-trial interval of 60 seconds.

Each trial began with the flower pot and rat separated by a barrier. Once the barrier was removed, the rat was given 60 seconds to find one reward. After the rat found the food reward, the barrier was replaced and the rat waited 10 seconds before starting the next trial. This stage was completed when the rat found 10 covered rewards. When the rats successfully completed day four of training, they were placed in the main experimental procedure.

## Exemplar Phase

After training in the digging task, a scent or digging medium was included as a new cue. Then rats were given a pretest using odor or digging medium as the relevant stimuli. Setup was

similar to that of day four of digging training in terms of barrier placement and trial times; however, now two pots were placed in the chamber with a barrier separating them from the rat, rather than one. Both flower pots were placed next to each other against the back wall of the testing area, but left one inch between them. For odor exemplars, the two flower pots had the same digging medium (e.g., clear beads; Creatology Mini-Beads), but each one had a different odor (e.g., citrus and lilac). For digging exemplars, the two flower pots had the same scent, (e.g., citrus), but they had different digging mediums (e.g., clear beads and blue gravel). In both cases, one scent (e.g., citrus) or digging medium (e.g., blue gravel; PetCo Blue Aquarium Gravel) was the relevant stimuli and contained a food reward. Both pots, however, had food dust placed underneath the digging medium to ensure that both pots had a grain pellet scent. The rats were given 60 seconds per trial to successfully choose the relevant stimuli. If the rat went over the 60-second time limit, the barrier was replaced and the trial was counted as a non-trial/missed trial. If the rat chose the incorrect pot, the trial was counted as incorrect. In order to complete the exemplars, the rat had to have chosen the relevant stimuli six times in a row. If the incorrect pot was chosen, the number of correct choices reset to zero.

## Cocaine Injections

Cocaine hydrochloride (generously supplied by National Insitutue of Drug Abuse) was dissolved in 0.9% sodium chloride dissolved in distilled water (Biofluids, Bioresource International). The rats in the cocaine group ($n$ = 20) were injected with 15 mg/kg cocaine intraperitoneally (i.p.) at a volume of 2 ml/kg for 10 consecutive days. This dose has been shown by Kavlias, Duffy, DuMars and Skinner (1988) to decrease the number of dopamine metabolites in the PFC after repeated administration. The study is seeking dosing regimen that results in changes in dopaminergic system. For this reason, 15 mg/kg is the optimal dose for

Experiment 1 (Kalivas et al., 1988). Eight of the rats receiving cocaine were pretreated with the $D_4$-receptor antagonist L-745,870 (Tocris Biosciences) dissolved in water with sodium chloride (0.9% concentration). Injections of L-745,870 were administered 20 minutes prior to testing at 0.1 mg/kg i.p. in 1-ml doses. This dose was chosen because optimal performance without motor effects was found between the doses of .05 mg/kg and .15 mg/kg (Zhang et al., 2004). The remaining cocaine rats ($n$ = 12) did not receive any pretreatment prior to testing.

A pool of control rats that was collected over Experiments 1, 2 and 3 ($n$ = 20 total) received i.p. injections of saline daily for 10 days. These control rats were used for control statistics in all experiments. One-way ANOVAs were run to confirm that no significant differences existed between the controls for each experiment. There were no statistical difference; therefore the data were pooled. All control rats were the same age, weight, and breed (Long-Evans) at the time of testing. A second control group ($n$ = 4) included rats receiving the same schedule of saline injections for 10 days; however, they also received a pretreatment of 0.1 mg/kg in a 1-ml dose of L-745,870 20 minutes prior to testing. This second saline-$D_4$ control is used in the statistical analysis of Experiments 1, 2 and 3.

Attentional Set-Shifting Task Phase

The rats were tested 24 hours after the last injection of cocaine or saline. The task was made up of seven stages: simple discrimination (SD), compound discrimination (CD), Reversal 1, intradimensional shift (IDS), Reversal 2, extradimensional shift (EDS), and Reversal 3. Each stage continued until the rat chose relevant stimuli correctly six times in a row. The simple discrimination stage was essentially the same set up as the exemplar phase in terms of there only being one different cue but with new scents and digging mediums. For example, in the SD stage, one of the stimuli was kept the same, digging or odor, depending on what the relevant stimuli

was. If digging medium was the relevant stimuli, then the smell (e.g., jasmine; all scents are Ashland Essential Oil purchased from Michael's Craft Store) was the same in both pots so that scent was an irrelevant stimulus. CD used the same set up as simple discrimination except a second stimulus was added. In this case, brown paper (S+) was still the rewarded stimuli; however, a vanilla scent cue was applied to one of the pots in addition to the jasmine scent, both of which are still irrelevant. Reversal 1 used the same setup as CD; however, the rewarded stimuli was switched, making white paper the reinforced stimuls (S+) and brown paper the unrewarded stimulus (S-).

The IDS used the same rules as the CD and Reversal 1. That is, the rat needed to learn that the digging medium was the relevant stimulus except that now a new digging medium was added (for example, multi-colored beads and clear beads; Creatology Pony Beads) while the scent remained the same. A second reversal, similar to the reversal of the third stage, switched the relevant digging medium again.

In this example, the first five stages of the task used a specific digging medium to predict reward. In the EDS, odor, which previously was irrelevant, was used to predict reward. This stage required the animal to forgo the previously learned rule of digging medium predicting reward, and adopt the new rule of odor predicting reward. For example, dark- and light-colored foam (Creatology Foam Sheets) is irrelevant to the now-rewarded scent of cinnamon. For the reversal stage, the rewarded scent was reversed and patchouli scent became relevant. Table 1 shows the pattern of the stages and the relevant dimensions.

Rats were counterbalanced for the switch from digging to odor or the odor to digging in the EDS. This switch was done to control for any potential preferences rats may have in terms of using odor or digging medium to signal reward.

Table 1. The 7-Stage ASST Task and Presentation of Various Stimuli. Each stage present a relevant and irrelevant dimension. Rewarded stimuli in the relevant dimension are denoted by S+ and unrewarded stimuli are denoted by S-.

| Dimensions | | | Examples of exemplars | | | |
|---|---|---|---|---|---|---|
| **Task** | **Relevant** | **Irrelevant** | **S+** | | **S-** | |
| **SD** | Medium | Odor | Brown Paper+ | Jasmine | White Paper | Jasmine |
| **CD** | Medium | Odor | Brown Paper+ | Vanilla | White Paper | Jasmine |
| **Rev 1** | Medium | Odor | White Paper+ | Jasmine | Brown Paper | Vanilla |
| **IDS** | Medium | Odor | Multi-color beads+ | Pine | Clear beads | Rose |
| **Rev 2** | Medium | Odor | Clear beads+ | Pine | Multi-color beads | Rose |
| **EDS** | Odor | Medium | Cinnamon+ | Dark foam | Patchouli | Light foam |
| **Rev 3** | Odor | Medium | Patchouli+ | Dark foam | Cinnamon | Light foam |

## Data and Data Analysis

Data that were collected for analysis included the number of trials required to reach criterion in the stages of interest for all three groups: R1, R2, R3, IDS, and EDS. Latency to respond (in seconds) was also collected per trial and analyzed in these stages. A one-way ANOVA was conducted on the number of trials required to reach criterion. Tukey post-hoc tests were conducted to understand where the impairments occurred. ANOVAs were also run on latency to respond for all groups in each stage. Tukey post-hocs were used to compare the times between groups. Levene's test of equality of variances was not significant; therefore, equal variance was assumed.

## Results

A one-way ANOVA with Tukey post-hoc was conducted on the reversal and attentional shift data for all three groups. Results showed that there was an overall difference in number of trials needed to reach criterion in the Reversal 1 ($F(3, 41) = 4.25$, $p = .011$) and Reversal 2 ($F(3, 41) = 6.62$, $p < .01$). There was no significance between any of the groups in Reversal 3. Tukey post-hoc analysis shows that there was a significant difference between the cocaine-treated rats

and the cocaine-$D_4$ group in Reversal 1 (*M* = 12.9, *SEM* = 1.55; *M* = 7.38, *SEM* = 0.51; *p* =.014). The cocaine-$D_4$ group performed similarly on Reversal 1 to both the saline control (*M* = 8.89, *SEM* = 0.83) and the saline-$D_4$ control group (*M* = 7.5, *SEM* = 0.87). On Reversal 2, cocaine group required significantly more trials than the saline control (*M* = 9.33, *SEM* = 0.77), the saline-$D_4$ control (*M* = 7.0, *SEM* = 2.0) and the cocaine-$D_4$ group (*M* = 8.25, *SEM* = 0.56; all *p*s <.01) to reach criterion (*M* = 13.4, *SEM* = 1.18). Figure 1 displays the number of trials needed to complete each reversal for all three groups.

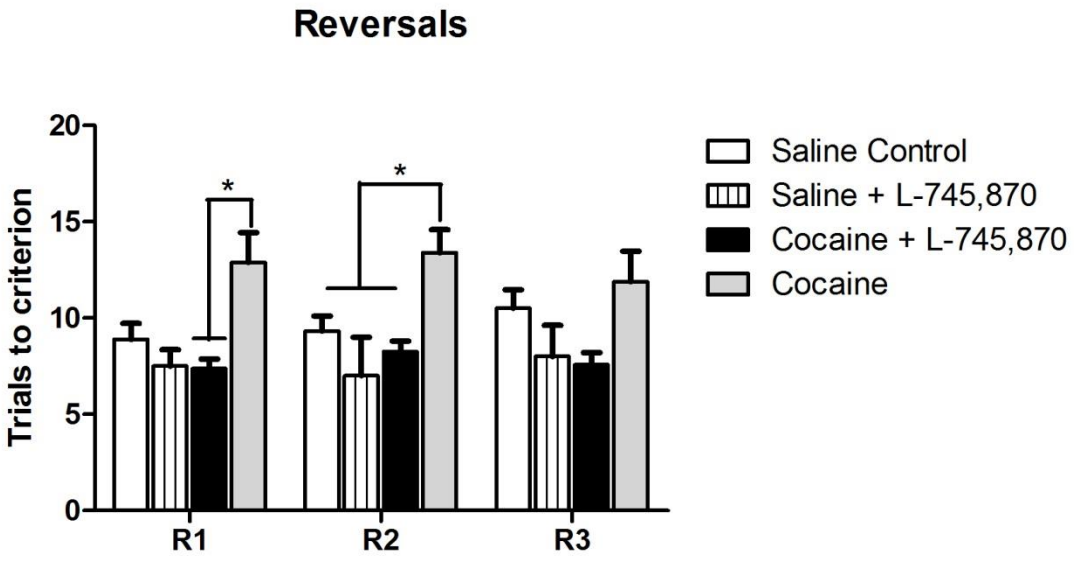


Figure 1. Mean (± SEM) trials required to reach criterion on the reversal stages for the saline control, saline-$D_4$ control, cocaine-$D_4$, and cocaine group. The cocaine group required significantly more trials to complete reversals one (p =.011) and two (p <.01). No significance was found in Reversal 3.

The one-way ANOVA also revealed that that there was a significant difference in the IDS ($F(3, 41) = 7.17$, $p < .01$) and the EDS ($F(3, 41) = 7.92$, $p < .01$). Tukey post-hoc analysis showed that the cocaine rats required significantly more trials to complete the IDS (*M* = 13.3, *SEM* = 1.23) than the saline control (*M* = 8.6, *SEM* = 0.71; *p* <.01), the saline-$D_4$ control group (*M* = 7.5, *SEM* = 0.65; *p* <.01) and the cocaine-$D_4$ group (*M* = 8.13, *SEM* = 0.479; *p* <.01). The same result is seen in the ED shift. The cocaine group needed 17.6 trials (*SEM* = 3.05) to complete

this stage compared to the saline control ($M$ = 8.56, $SEM$ = 0.532), the saline-$D_4$ control ($M$ = 6.75, $SEM$ = 0.75) and the cocaine-$D_4$ group ($M$ = 8.25, $SEM$ = 0.526, all $p$s <.01). Figure 2 displays the trials to criterion in attentional shifts.

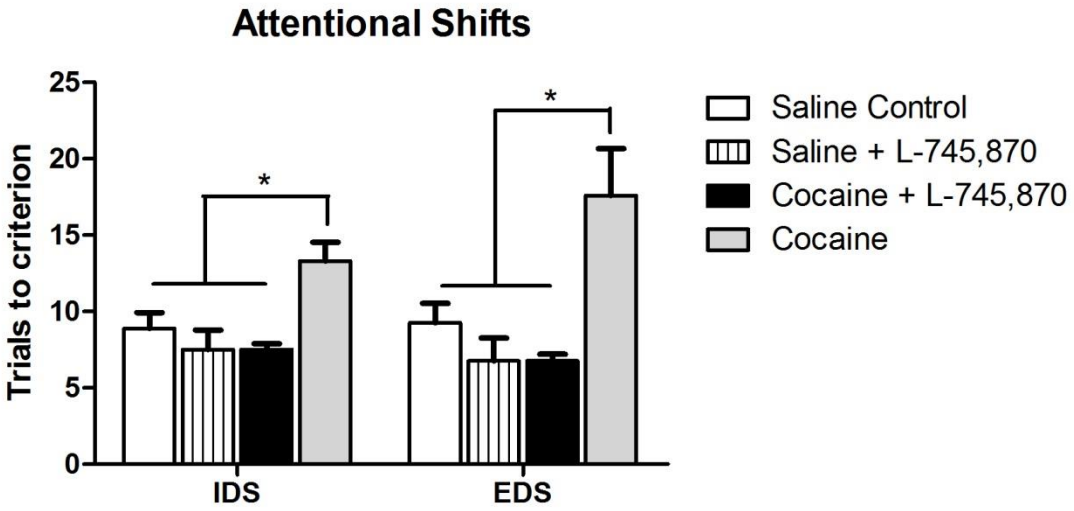


Figure 2. Mean (± SEM) trials to criterion for attentional shifts, IDS and EDS for all four groups. The cocaine group required significantly more trials to complete both stages compared to the two control groups and the $D_4$ cocaine group. All $p$s <.05.

Any differences in the ability to acquire the task were assessed in a one-way ANOVA on the SD and CD trials to criterion. A significant difference was found in the SD ($F$(3,41) = 3.35, $p$ = .029) only between the cocaine group and the saline-$D_4$ control group ($p$ =.046). Since the cocaine group showed an overall impairment on both the reversals and the attentional shifts, the cocaine group requiring 10.1 trials ($SEM$ = 1.49) to reach criterion compared to the saline-$D_4$ control group's 6.75 trials ($SEM$ = 0.48) is to be expected. Often times an impairment in acquisition phases is also seen in later stages. An ANOVA was also conducted on the latency to respond for each group although that yielded no significant differences between groups. The latencies for reversals and shifts are depicted in figures 3 and 4 respectively.

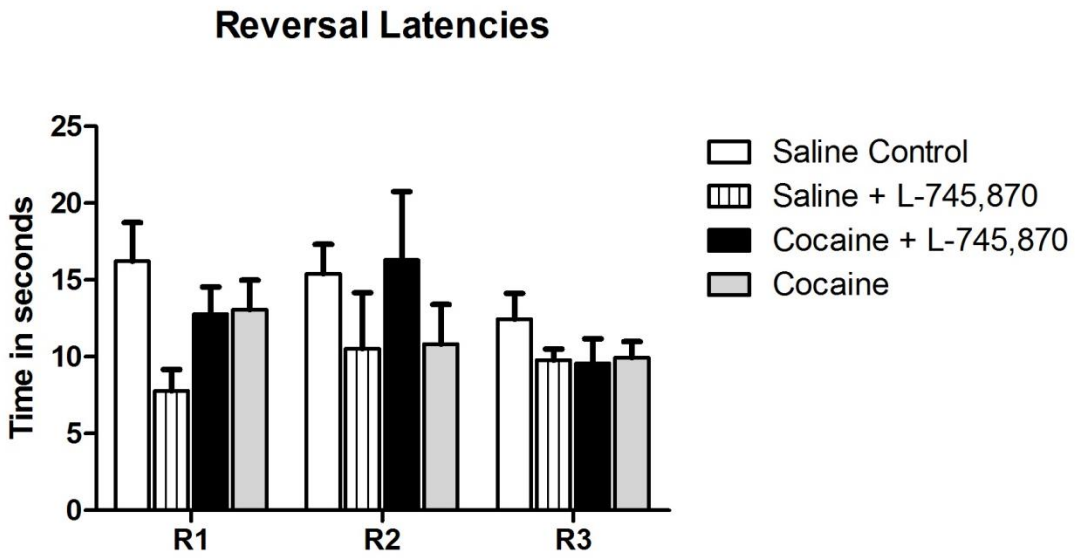


Figure 3. The mean (± SEM) time in seconds required to complete the reversal stages for all four groups. There was no significant difference between any of the groups in the reversal stages.

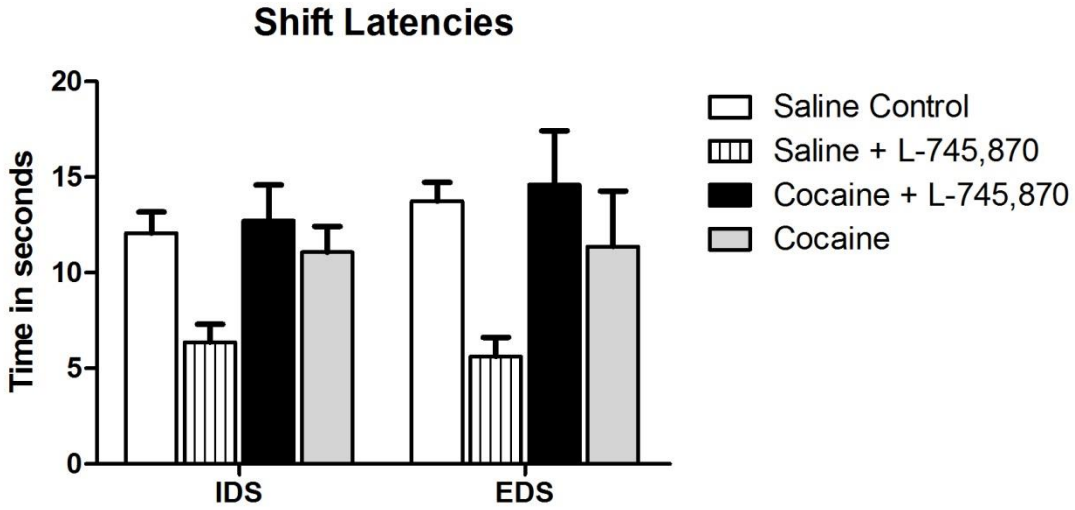


Figure 4. The mean (± SEM) time in seconds required to complete the attentional shift stages for all four groups. There was no significant difference between any of the groups in either the IDS or EDS.

# CHAPTER 3

# EXPERIMENT 2: METHODS AND RESULTS

Experiment 2 focuses on amphetamine's effect on behavioral flexibility. Since cocaine administration impairs ASST performance, the experiment was repeated with amphetamine, another potent stimulant of abuse, to see if similar impairments would be found. Both drugs inhibit dopamine transporter (DAT) to increase dopamine availability in the synapse, but amphetamine also indirectly stimulates dopamine release. Based on this difference in pharmacological actions, differences in ASST performance could be expected. Reports have shown that amphetamine use targets reversal learning, also found in cocaine use, but not attentional shifts, which is found in cocaine use. This is possibly due to the fact that each stage is mediated by different frontal cortices. This experiment evaluates the effect of amphetamine on reversal-learning impairment within the present experimental design and also evaluates prospects for impairment reversal. The $D_4$ receptors are specific to the PFC, and therefore targeting them should be able to improve performance in all stages despite each stage being mediated by different frontal cortices. Experimental methods for food restriction, learning to dig, exemplars and ASST are the same as Experiment 1.

## Amphetamine Injections

Amphetamine (generously supplied by NIDA) was dissolved in a 0.9% sodium chloride water solution (Biofluids, Bioresource International). The rats in the amphetamine group ($n$ = 16) were injected with 2 mg/kg amphetamine i.p. at a volume of 2 ml/kg for 10 consecutive days. This dose was chosen because it has been shown to increase dopamine levels in the striatum (Zetterström, Sharp, Marsden & Ungerstedt, 2006). The pooled control rats ($n$ = 20) received equivalent i.p. injections of saline daily for 10 days as did the saline-L-745,870 control rats.

Half of the amphetamine rats ($n$ = 8) were pretreated with the $D_4$ receptor antagonist L-745,870 (Tocris Biosciences) dissolved in a 0.9% sodium chloride water solution. Injections were administered 20 minutes prior to testing at 0.1 mg/kg i.p. in 1-ml doses. The saline-L-745,870 control group received the same dose 20 minutes prior to testing.

## Data and Data Analysis

Data that were collected for analysis included the number of trials required to reach criterion in the stages of interest for all three groups: R1, R2, R3, IDS, and EDS. Latency to respond (in seconds) was also collected per trial and analyzed in these stages. A one-way ANOVA was conducted on the number of trials required to reach criterion and latency. Tukey post-hoc tests were conducted to see where the impairments occurred. ANOVAs were also run on latency to respond for all groups in each stage. Tukey post-hocs were used to compare the times between groups. Levene's test of equality of variances was not significant, therefore, equal variance was assumed.

## Results

A one-way ANOVA with Tukey post-hoc analysis was conducted on the reversal and attentional shift data for all three groups. Results showed that there was an overall difference in the number of trials needed to reach criterion in Reversal 1 ($F(3, 30) = 27.81$, $p < .01$), Reversal 2 ($F(3, 30) = 13.12$, $p < .01$) and Reversal 3 ($F(3, 29) = 11.71$, $p < .01$). Tukey post-hoc analysis shows that there was a significant difference among the three groups in all three reversal stages (all $p$s <.01). The saline control group and the amphetamine-$D_4$ group performed approximately equally in Reversal 1 ($M$ = 9.25, $SEM$ = 1.08 to $M$ = 9.75, $SEM$ = .98), Reversal 2 ($M$ = 9.42, $SEM$ = 0.82 to $M$ = 10.88, $SEM$ = 0.61) and Reversal 3 ($M$ = 9.67, $SEM$ = 1.06 to $M$ = 9.57, $SEM$ = 0.99). Amphetamine groups required significantly more trials than both groups to reach

criterion in each reversal (*M* = 19, *SEM* = 1.30, *M* = 16.38, *SEM* = 1.34, and *M* = 17.13, *SEM* = 1.29, respectively). Tukey post-hoc results also show that the saline-$D_4$ control group did not differ significantly from the saline control group or the amphetamine-$D_4$ group in any of the reversal stages (R1: *M* = 7.5, *SEM* = 0.87, R2: *M* = 7.0, *SEM* = 1.0, and R3: *M* = 6.75, *SEM* =1.5). The only differences seen are between the saline-$D_4$ control group and the amphetamine-only group in all three reversal stages (all *p*s <.01). Figure 5 displays the number of trials needed to complete each reversal for all three groups.

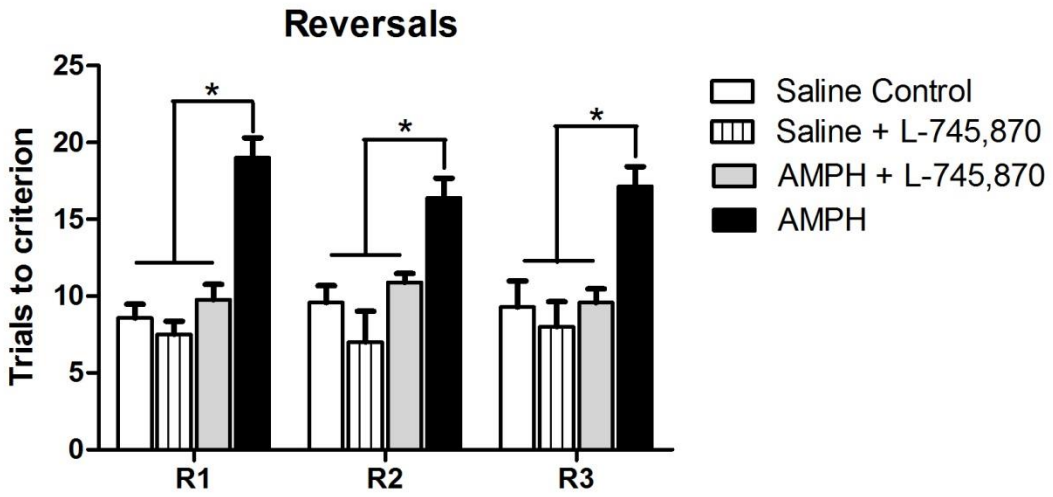


Figure 5. Mean (± SEM) trials to criterion for reversals one, two and three. The amphetamine group required significantly more trials to complete each reversal than the saline control, saline-$D_4$ control, and the amphetamine-$D_4$ group. Each reversal was significant at $p$ <.01.

The one-way ANOVA also revealed that no significant difference appeared in any group on either IDS or EDS. All groups performed similarly to each other in the IDS (saline control: *M* = 7.33, *SEM* = 0.67; saline-$D_4$ control: *M* = 7.5, *SEM* = 1.29; amphetamine-$D_4$: *M* = 10.1, *SEM* = 0.61; amphetamine: *M* = 7.63, *SEM* = 0.89 trials) and the EDS (saline control: *M* = 8.00, *SEM* = 0.49; saline-$D_4$ control: *M* = 6.75, *SEM* = 0.43; amphetamine-$D_4$: *M* = 9.71, *SEM* = 0.79; amphetamine: *M* = 8.2, *SEM* = 0.95 trials; see Figure 6). A one-way ANOVA on the acquisition

stages, SD and CD, revealed no significant difference between any of the groups. This implies that all groups were able to acquire the attentional set in the first two stages.

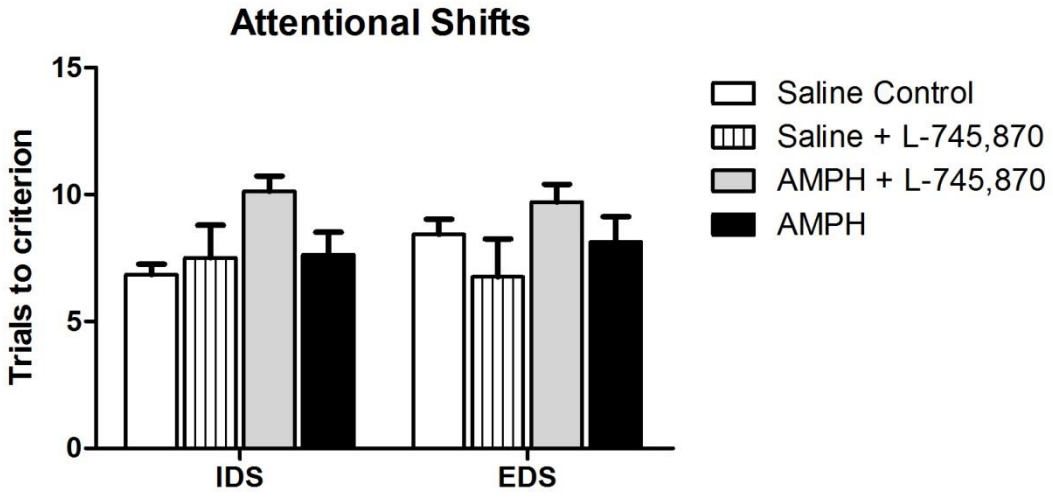


Figure 6. Mean (± SEM) trials to criterion in the attentional shift stages, IDS and EDS. There was no significant difference among any of the groups on IDS or EDS performance.

Results from a one-way ANOVA on latency to respond in reversals and shifts showed that there was a significant difference in response times in Reversal 1 ($F(3,26)$ =5.04, $p$<.01), Reversal 2 ($F$(3, 25) = 4.82, $p$ <.01), Reversal 3 ($F$(3, 25) = 3.72, $p$ =.026), IDS ($F$(3, 25) = 7.95, $p$ <.01) and EDS ($F$(3, 26) = 3.49, $p$ =.032). Tukey post-hoc tests show that these significant differences are found in the amphetamine group when compared to the amphetamine-$D_4$ group. The amphetamine-$D_4$ group performed faster than the amphetamine group in Reversal 1 ($M$ = 8.96, $SEM$ = 2.39 to $M$ = *17*.52s, $SEM$ = 1.90; $p$ =.027), Reversal 2 ($M$ = 7.92s, $SEM$ = 1.42 to $M$ = 18.36s, $SEM$ = 3.13; $p$ <.01), and Reversal 3 ($M$ = 6.08s, $SEM$ = 0.41 to $M$ = 15.97, $SEM$ = 1.96; $p$ =.019). There was a significant difference in latency between the amphetamine-$D_4$ group and the amphetamine group in the IDS only ($M$ = 7.92s, $SEM$ = 1.52 to $M$ = 14.61, $SEM$ = 0.99; $p$ <.01). Neither of the $D_4$-treated groups, saline or amphetamine, was significantly different from the saline controls in terms of latency. The saline-$D_4$ control did differ significantly from

the amphetamine alone group in reversal 1 ($M$ = 7.78s, $SEM$ = 1.40; $p$ =.043), IDS ($M$ = 6.38s, $SEM$ = 0.92; $p$ <.01) and EDS ($M$ = 5.62s, $SEM$ = 0.99; $p$ =.039). Some rats in all three groups were missing latency data due malfunctioning equipment. These missing data were excluded from the latency statistics. This accounts for the differences in degrees of freedom. Figures 7 and 8 display the latency data for each group.

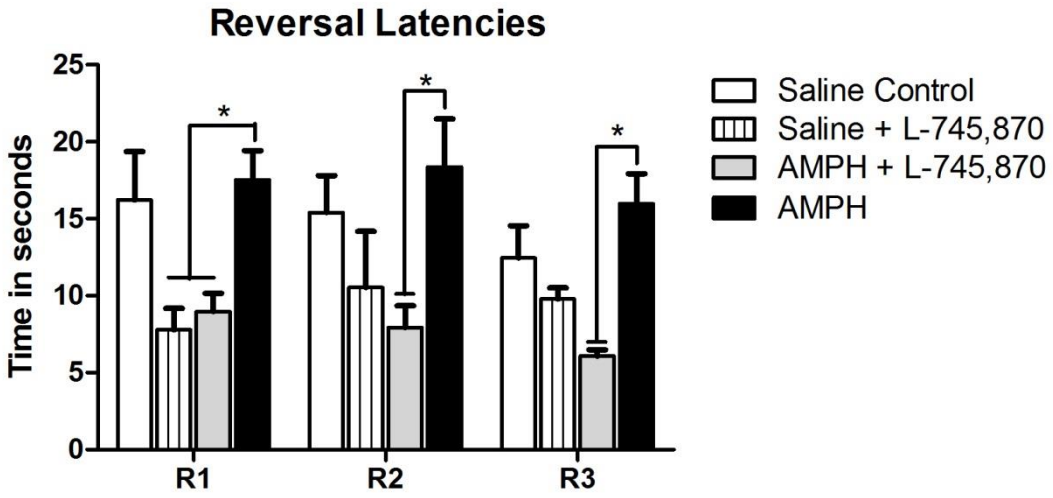


Figure 7. Mean (± SEM) time in seconds required to complete each reversal stage. The amphetamine group required more time (in seconds) to complete each reversal stage than the amphetamine-$D_4$ group (each $p$ significant at <.05). In Reversal 1, the saline-$D_4$ control also performs significantly faster than the amphetamine group ($p$ =.043)

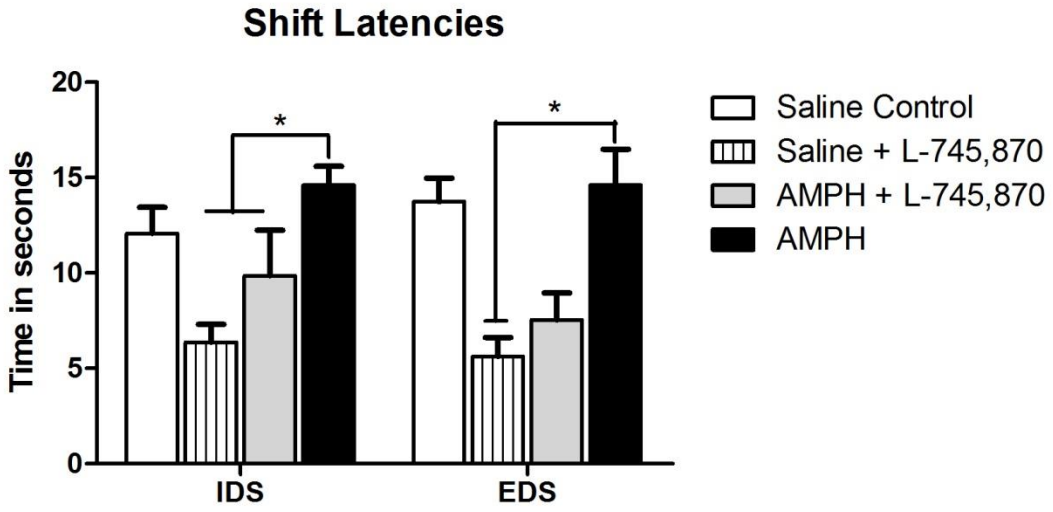


Figure 8. Mean (± SEM) time in seconds required to complete each attentional shift stage, IDS and EDS. The amphetamine group required significantly more time in the IDS than both L-745,870-treated groups (both *p*s <.01). In the EDS, only the saline-$D_4$ control group performed significantly faster than the amphetamine group ($p$ =.039)

# CHAPTER 4

# EXPERIMENT 3: METHODS AND RESULTS

Experiment 3 examines the effect of quinpirole, a $D_2/D_3$ agonist, on ASST performance. Evidence reveals a clear link between cocaine and $D_2$ receptors. During cocaine administration, dopamine release floods predominantly $D_2$ receptors. Dopamine binding to these specific receptors promotes reinforcing effects similar to cocaine. Rats previously trained to self-administer cocaine will self-administer $D_2$-receptor agonists (Caine, Negus, Mello & Bergman, 1999). In cocaine abusers, $D_2$-receptor downregulation is observed well into abstinence (Volkow, Fowler & Wang, 2003). Animals that self-administered cocaine saw a similar $D_2$-receptor downregulation within a week of self-administration and that downregulation lasted up to a year (Nader et al., 2006). Based on this evidence, it seemed necessary to target $D_2$-receptors specifically. In addition to its targeted effect on $D_2/D_3$ receptors, quinpirole was used to examine the effect of dopamine alone. Cocaine and amphetamine can also alter levels of serotonin and norepinephrine during use. Therefore, the results seen in Experiments 1 and 2 could be due not only to dopamine dysregulation, but also serotonin and norepinephrine. For this reason, quinpirole was chosen to look more specifically at dopamine's role in ASST performance. Experimental methods for food restriction, learning to dig, exemplars and ASST are the same as in Experiment 1.

## Quinpirole Injections

Quinpirole (Tocris Biosciences) was dissolved in a 0.9% sodium chloride water solution (Biofluids, Bioresource International). The rats in the quinpirole group ($n$ = 16) were injected with 0.2 mg/kg quinpirole i.p. at a volume of 2 ml/kg for 10 consecutive days. This dose was chosen because it fell between the two effective doses tested in Boulougouris, Castañe and

Robbins (2008) in a visual discrimination task and increased perseverative responding in reversal trials. Pooled rats ($n$ = 12) received equivalent i.p. injections of saline daily for 10 days as did the saline-L-745,870 control rats.

Half of the 10-day quinpirole rats ($n$ = 8) were pretreated with the D4 receptor antagonist L-745.870 (Tocris Biosciences) dissolved in a 0.9% sodium chloride water solution. Injections were administered 20 minutes prior to testing at 0.2 mg/kg i.p. in 1-ml doses. The saline-L-745,870 control group received the same dose 20 minutes prior to testing.

## Data and Data Analysis

Data that were collected for analysis included the number of trials required to reach criterion in the stages of interest for all three groups: R1, R2, R3, IDS, and EDS. Latency to respond (in seconds) was also collected per trial and analyzed in these stages. A one-way ANOVA was conducted on the number of trials required to reach criterion. Tukey post-hoc tests were conducted to see where the impairments occurred. ANOVAs were also run on latency to respond for all groups in each stage. Tukey post-hoc tests were used to compare the times between groups. Levene's est of equality of variances was not significant; therefore, equal variance was assumed.

## Results

A one-way ANOVA between saline controls, saline-$D_4$ control, quinpirole-$D_4$, and quinpirole group revealed a significant difference among the groups in Reversal 1 ($F(3,39) = 5.59$, $p < .01$.) and Reversal 2 ($F(3,39) = 3.28$, $p = .032$), although there was no significance in Reversal 3. Tukey post-hoc analysis reveals that the quinpirole-$D_4$ group required significantly more trials to complete Reversal 1 ($M$ = 14.5, $SEM$ = 1.5) when compared to the saline control ($M$ = 9.3, $SEM$ = .83; $p < .01$), the saline-$D_4$ control ($M$ = 7.5, $SEM$ = .65; $p = .011$), and the

quinpirole group ($M$ = 9.13, $SEM$ = .69, $p$ =.019). In Reversal 2, despite showing a significance in the one-way ANOVA, the Tukey post-hoc tests revealed no significance between any of the groups. The quinpirole-$D_4$ approached significance when compared to the quinpirole only group ($p$ =.061) and the saline-$D_4$ control group ($p$ =.051). Figure 9 depicts the mean trials to criterion for the reversals.

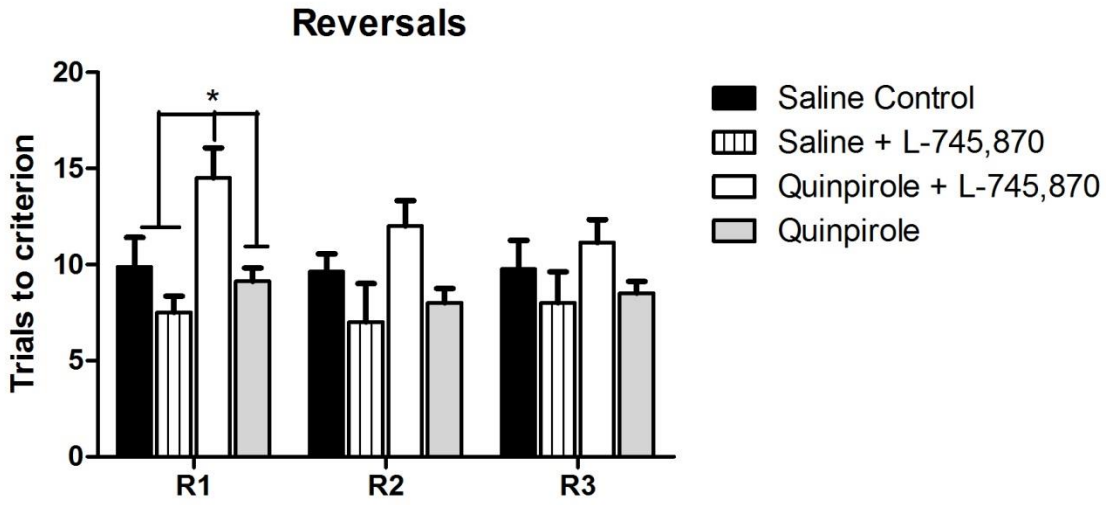


Figure 9. Mean (± SEM) trials to criterion in the three reversal stages for the saline control, saline-$D_4$ control, quinpirole-$D_4$, and quinpirole group. The quinpirole-$D_4$ group required significantly more trials to complete Reversal 1 compared to the quinpirole group, saline control and saline-$D_4$ control (each $p$s significant at <.05). Reversal 2 was significant overall, however, no group displayed significance over another.

According to the one-way ANOVA, there was a significant difference between groups in both attentional shift stages, IDS ($F$(3, 39) = 5.76, $p$ <.01) and EDS ($F$(3, 38) = 21.99, $p$ <.01). Tukey post-hoc tests for the IDS indicate that the quinpirole group ($M$ = 12.75, $SEM$ = 1.08) required significantly more trials to reach criterion than the saline control ($M$ = 8.6, $SEM$ = .71, $p$ <.01), saline-$D_4$ control ($M$ = 7.5, $SEM$ = .65, $p$ =.017) and the quinpirole-$D_4$ group ($M$ = 8.13, $SEM$ = .48, $p$ <.01). The quinpirole group also was significantly worse on the EDS trials ($M$ = 18.5, $SEM$ = 1.59) than the saline control ($M$ = 9.15, $SEM$ = .64, $p$ <.01), saline-$D_4$ control ($M$ = 6.75, $SEM$ = .75, $p$ <.01) and the quinpirole-$D_4$ group ($M$ = 9, $SEM$ = .72, $p$ <.01).

Performance on attentional shifts can be seen in Figure 10. A one-way ANOVA on the acquisition stages, SD and CD, revealed no significant difference between any of the groups. This implies that all groups were able to acquire and learn the attentional set in the early stages.

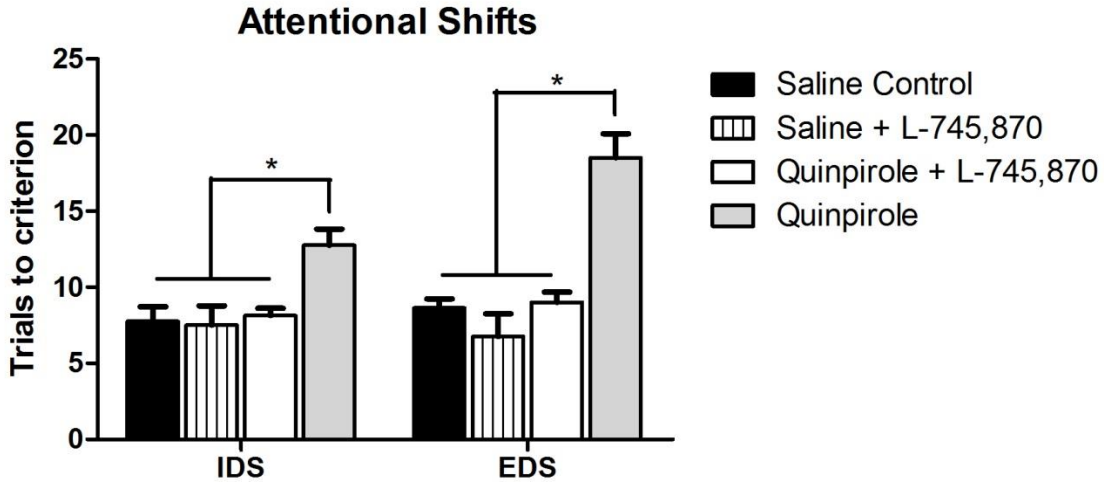


Figure 10. Mean (± SEM) number of trials to criterion for attentional shifts, IDS and EDS. The quinpirole group required significantly more trials to complete each of these stages compared to the saline control, saline-$D_4$ control, and quinpirole-$D_4$ group. All *p*s <.05.

A second one-way ANOVA was conducted on the response latencies from all four groups in reversals and attentional shifts. Results from a one-way ANOVA on latency to respond in reversals and shifts showed that there was a significant difference in response times in Reversal 3 ($F(3,25) = 3.28$, $p = .04$) and EDS ($F(3,25) = 9.07$, $p < .01$). Tukey post-hoc tests of the third reversal show that these significant differences are found in the saline group when compared to the quinpirole-$D_4$ group. The saline group required longer time to respond per trial than the quinpirole-$D_4$ group in reversal 3 ($M$ = 12.45s, $SEM$ = 2.11 to $M$ = 6.09s, $SEM$ = 0.434; $p = .032$). In the EDS, Tukey post-hoc tests showed that saline rats required more time in this stage compared to all groups. The saline-$D_4$ control ($M$ = 5.62s, $SEM$ = .98), quinpirole-$D_4$ group ($M$ = 7.58s, $SEM$ = 1.23) and quinpirole group ($M$ = 8.02, $SEM$ = .87) all performed

faster that the saline control on the EDS (all *p*s <.01). Figures 11 and 12 display the latency data for each group.

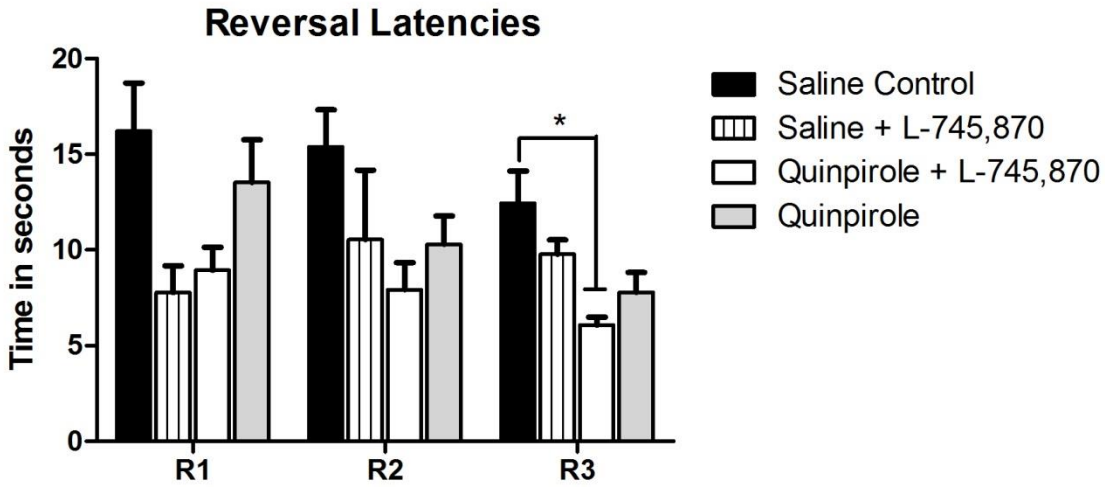


Figure 11. Mean (± SEM) time in seconds for all three reversals. The saline control required significantly more time to respond than the quinpirole-$D_4$ group in Reversal 3 ($p$ =.032). All other reversals were not significant.

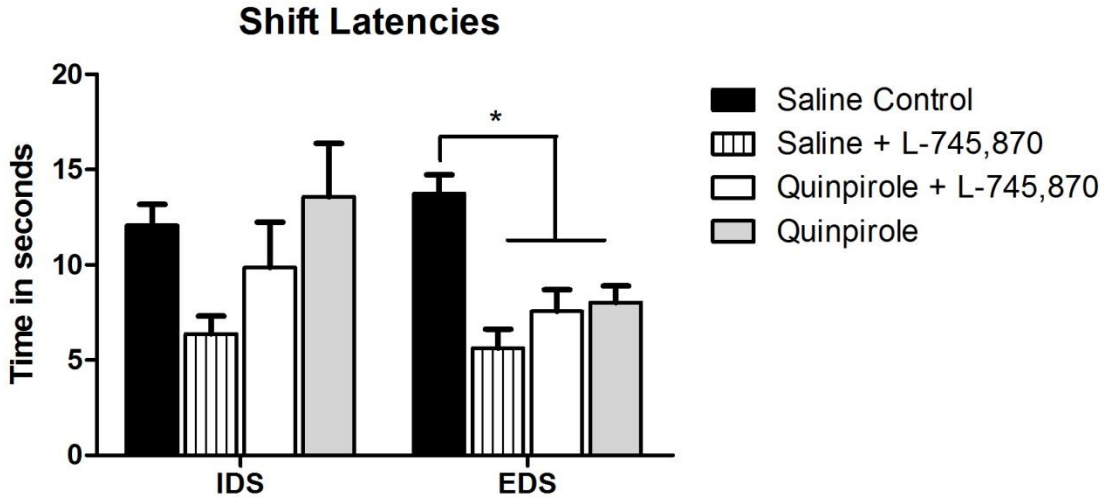


Figure 12. Mean (± SEM) time in seconds for attentional shift stages IDS and EDS. The saline control required significantly more time to respond than all three groups in the EDS (all *p*s <.01). IDS latency did not reveal any significance differences among groups.

# CHAPTER 5

## SUPPLEMENTARY DATA: METHODS AND RESULTS

Supplementary Experiments 4 and 5 were conducted post-hoc to amplify the outcomes from Experiments 1, 2 and 3. Theories regarding how deficits in the ASST arise involve various neurotransmitters; most of which are affected by amphetamine, cocaine and quinpirole. For example, at certain doses, cocaine is thought to act on serotonin release; therefore, observing serotonin's effect on ASST performance is important. These supplementary experiments were conducted to support some of the conclusions made in the discussion section. Most of the data presented in both experiments is already well documented from previous studies. They were tested in an experimental method similar to that of Experiments 1, 2 and 3 in order to make direct comparisons.

Supplementary Experiment 4 was conducted because of a connection between $D_4$ receptors and NMDA receptor activity. The effect of NMDA receptor blockade has been shown to have an effect on cognitive performance (Stefani & Moghaddam, 2003). When $D_4$ receptors are activated, NMDA receptors internalize and subsequently impair performance (see Discussion). MK-801 is an NMDA receptor antagonist that would mimic the effects of NMDA receptor internalization. Ideally, the results from this study would support the hypothesis that $D_4$ receptor activation would decrease availability of the NMDA receptors, and $D_4$ receptor blockade would in turn improve performance.

The effect of serotonin (5-HT) depletion on reversal learning is also well documented (Clarke et al., 2005; Clarke, Walker, Dalley, Robbins & Roberts, 2007). Cocaine, amphetamine and quinpirole can act on serotonin at certain doses. Supplementary Experiment 5 was conducted to make a comparable group for the drug-tested rats. The experimental methods are the same as in previous experiments. However, rather than ten days of injections, animals were

tested 24 hours after exemplar phase and received a pharmacological intervention 20 minutes prior to testing.

## MK-801 Injections

MK-801 (Tocris Biosciences) was dissolved in a 0.9% sodium chloride water solution (Biofluids, Bioresource International). The rats in the MK-801group ($n$ = 8) were injected with 0.1 mg/kg MK-801 i.p. at a volume of 2 ml/kg 20 minutes prior to testing. Pooled control rats ($n$ = 20) received equivalent i.p. injections of saline.

## Attentional Set-Shifting Task Phase

Initially, the rats were meant to run through all seven stages of the ASST task, however, the effect of the MK-801 prevented the rats from completing the SD stage. Rats were allowed to complete 50 trials in the SD stage before being pulled from testing. Instead of a one-way ANOVAs observing the effect of the drug on performance by stage, the MK-801 rats were compared to their own performance on the exemplar test 24 hours prior to testing on the drug. The exemplar and SD stage are the same test (one perceptual dimension remains the same while the other dimension signals reward); however, the exemplar and SD stage use different stimuli in testing. This prevents any familiarization with the scent or medium from influencing responses. Statistical analysis used paired t-tests to compare the latency to respond and the percentage of correct choices off (exemplar) and on (ASST) MK-801.

## Results

A paired samples t-test comparing the percent correct choice in the exemplar task and the ASST task was conducted. Results indicate that rats on the MK-801 during performance in the ASST were significantly choosing the correct option less often ($M$ = 44.6%, $SEM$ = 3.54) compared to the exemplar performance [($M$ = 83.7%, $SEM$ = 4.34, $t(7) = -6.48$, $p < .01$, Figure

13)] A second paired samples t-test comparing mean latency to respond in the exemplar task to the ASST task showed that MK-801 significantly decreased the latency to respond (*M* = 14.1 seconds, *SEM* = 2.7) compared to the same rats off the drug in the exemplars [(*M* = 29 seconds, *SEM* = 3.38, *t*(7) = -5.1, *p* <.01, Figure 14)].

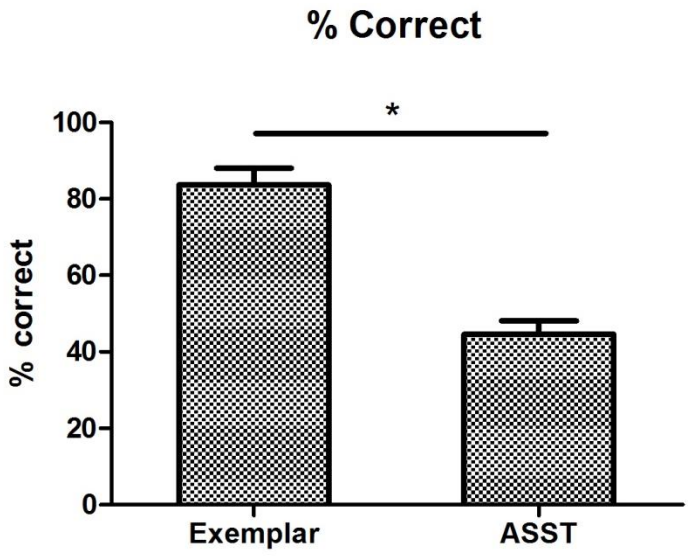


Figure 13. Mean (± SEM) percentage of correct responses in rats during the exemplar (off-drug) and the ASST (on-drug). MK-801 pretreatment significantly reduced the correct choice percentage compared to the exemplars tested off-drug (*p* <.01).

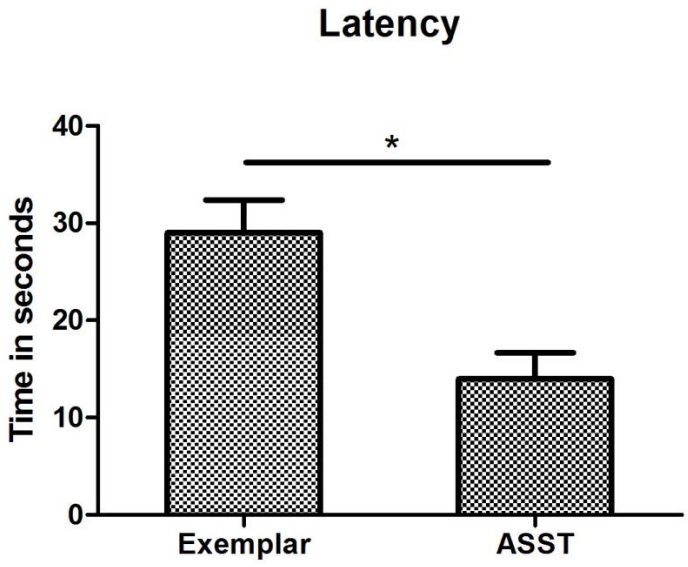


Figure 14. Mean (± SEM) time in seconds to respond in the exemplar (off-drug) and the ASST (on-drug). MK-801 pretreatment significantly reduced their latency to respond compared to when tested off-drug (*p* <.01).

## Serotonin Agonist and Antagonist Injections

The serotonin rats ($n$ = 8) received injections of both the serotonin agonist (serotonin hydrochloride, Alfa Aesar) and the serotonin antagonist (Methysergide maleate, Tocris Biosciences) prior to testing on the ASST task. Rats were counterbalanced for which treatment they received first to control for an effect of retesting. Half of the rats ($n$ = 4) received an injection of serotonin hydrochloride (0.1 mg/kg dissolved in a 0.9% sodium chloride water solution) 20 minutes prior to testing. Forty-eight hours later these rats received an injection of methysergide maleate (0.1 mg/kg dissolved in 0.9% sodium chloride water solution) 20 minutes prior to testing. The second half of the rats received the injections in the opposite order. The ASST was also counterbalanced so that each treatment involved an attentional switch of digging to odor and odor to digging.

## Data and Data Analysis

Data that was collected for analysis included the number of trials required to reach criterion in the stages of importance for all three groups: R1, R2, R3, IDS, and EDS. Latency to respond (in seconds) was also collected per trial and analyzed in these stages. A one-way ANOVA was conducted on the number of trials required to reach criterion. Tukey post-hoc tests were conducted to see where the impairments occurred. Levene's test of equality of variances was not significant, therefore, equal variance was assumed.

## Results

Results from the one-way ANOVAs on the agonist data showed no significant difference on performance in either the reversals or the attentional shifts. One-way ANOVAs on the antagonist data showed a significant difference between the groups on Reversal 1 ($F(2, 35) = 22.787$, $p < .01$), Reversal 2 ($F(2, 34) = 21.886$, $p < .01$), and Reversal 3 ($F(2, 33) = 3.534$, $p$

<.041). According to Tukey post-hoc analysis, the antagonist group required significantly more trials to complete Reversal 1 ($M$ = 19.5, $SEM$ = 1.69, $p$ <.01) and Reversal 2 ($M$ = 18.9, $SEM$ = 1.807, $p$ <.01), and when compared to controls ($M$ = 9.3, $SEM$ = 0.788, $M$ = 9.4, $SEM$ = 0.688) and the agonist group ($M$ = 8.86, $SEM$ = 1.164 and $M$ = 9.00, $SEM$ = 1.095) in those stages. Figure 15 depicts mean trials in reversal stages. In Reversal 3, the Tukey post-hoc tests show that the antagonist group only required more trials than the control group ($M$ = 16.57, $SEM$ = 1.131 and $M$ = 10.71, $SEM$ = 0.873; $p$ =.034). The antagonist group performed equally to controls and the agonist group on attentional shifts. No significant difference was found in the number of trials needed to reach criterion (Figure 16). A one-way ANOVA looking at SD and CD trials to criterion revealed a significant difference between the three groups ($F$(2,34) = 3.51, $p$ =.042) but Tukey post-hoc analysis did not show any significance.

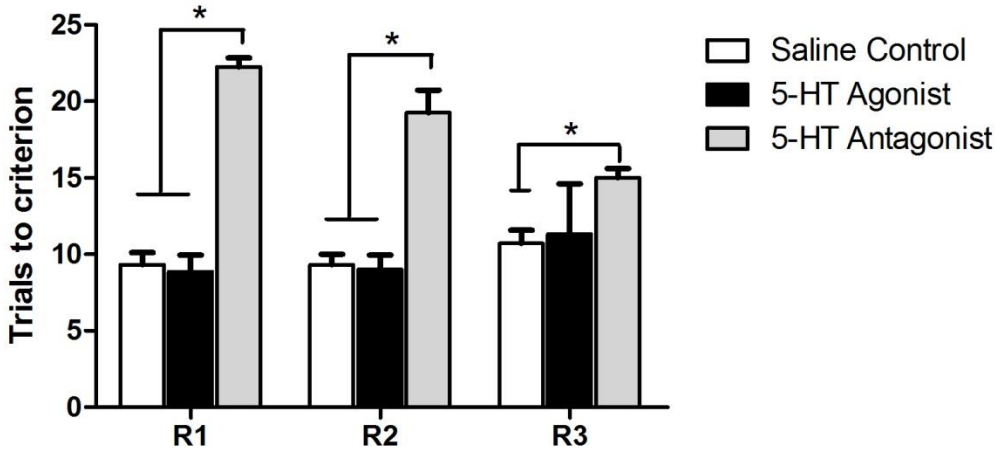


Figure 15. Mean (± SEM) trials needed to reach criterion in all three reversals. Rats tested on the 5-HT antagonist were significantly impaired compared to the saline controls and 5-HT agonist counterparts (both $p$s <.01). In Reversal 3, the 5-HT agonist group was significant only to the saline control ($p$ =.04).

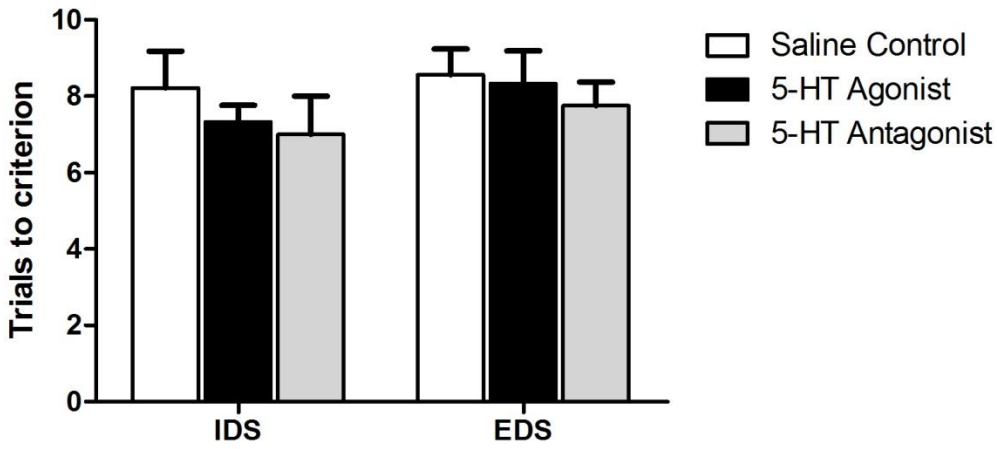


Figure 16. Mean (± SEM) trials needed to reach criterion in both attentional shift stages, IDS and EDS. No group was significantly different from the others in either attentional shift stage.

# CHAPTER 6

# GENERAL DISCUSSION

The results have shown that various drugs of abuse impair ASST performance and that those impairments are potentially neurotransmitter and/or structurally dependent. Cocaine-treated rats showed a general impairment of reversal and attentional shifts requiring more trials to complete each stage. Amphetamine-treated rats were significantly impaired only on reversal stages. Quinpirole rats, like cocaine-treated rats, were significantly impaired on the attentional shift stages, IDS and EDS. More interestingly, $D_4$ receptor antagonist, L-745,870, was able to reverse the impairments caused by each drug. In the case of quinpirole, however, blocking $D_4$ receptors revealed a reversal impairment not seen in the quinpirole-only group. Each experiment is discussed in greater detail below.

## Cocaine

Results show that cocaine rats had impairments in both attentional shifts and all but the third reversal stage. Moreover, the impairment seen in the cocaine group was reversed with the pretreatment of a $D_4$ antagonist. This finding is consistent with other work specifically when looking at reversal learning impairments (Calu et al., 2007; Schoenbaum et al., 2004;Stalknaker et al., 2009). There are varied accounts on the effect of cocaine on attentional shifts. Human research on cocaine abusers often report impairments in the WCST stages that require dimensional shifts (Colzato, Huizinga & Hommel, 2009; Ersche et al., 2006; Woicik et al., 2011). In animal research, both impairments (Garavan et al., 2000) and improvements (Black et al., 2006) in the EDS have been reported. While cocaine's main effect is blocking the dopamine transporter preventing reuptake of dopamine, it also blocks the reuptake of norepinephrine and serotonin (Nestler, 2005). All three neurotransmitters are known to have differing effects on performance in the ASST. For example, decreases in norepinephrine impair EDS, decreases in

serotonin impair reversals (as seen in Supplementary Experiment 5), and dopamine has been shown to either improve or impair EDS performance depending on the location of the depletion (Robbins & Roberts, 2007; Tait et al., 2007; Lapiz, Bondi & Merilak, 2006). Serotonin transporter binding in cocaine users was lower than controls (Little et al., 1998). This suggests that it is possible that rats tested after cocaine administration experienced a downregulation of serotonin receptors in addition to dopamine receptors. In the Supplementary Experiment 5, the reversals show a clear impairment in only reversals during serotonin depletion – an effect possibly seen in the cocaine-treated rats as well. In Experiment 1, $D_4$ antagonist pretreatment improved all impairments previously seen in the cocaine-only rats. This suggests that repeated doses of cocaine in this study may not target one particular stage but rather all stages to varying degrees.

Amphetamine

The study presented here shows that repeated doses of amphetamine result in significant impairments in reversal learning but no effect in attentional shifts. Our findings are consistent with previous work regarding amphetamine's ability to impair reversal learning (Fletcher, Tenn, Rizos, Lovic & Kapur, 2005; Idris, Repeto, Neill & Large, 2005; Ridley, Haystead & Baker, 1981). In addition, we also show that the $D_4$ receptor-specific antagonist, L-745,870, is able to reverse the impairments caused by repeated doses of amphetamine.

Amphetamine appears to cause deficits similar to those of OFC lesions in that they primarily affect reversal learning (Dias, Robbins & Roberts, 1997; Schoenbaum et al., 2004). However, repeated exposure to amphetamine seems to have no effect on the attentional shifts that have been shown to be mediated by the mPFC in rats (Birrell & Brown, 2000). This suggests that repeated doses of amphetamine primarily affect the OFC or possibly a part of the

fronto-striatal network which the OFC depends on for functional stimulus-response learning. This idea is suggested by Izquierdo et al. (2010) in their study using a binge regimen (four injections at 2-h intervals) of mAMPH. They found that mAMPH impaired early reversal learning and there also was a significant reduction in dopamine binding in the dorsal and ventral subdivisions of the caudate putamen (Izquierdo et al., 2010). They suggest that mAMPH decreases dopamine binding in the striatum, which in turn affects the OFC. This idea is supported by Castañe, Theobold and Robbins (2010). Lesions to the dorsomedial striatum produced significant impairments in reversal learning, namely, perseverative errors. Similar results are seen also in monkeys with medial striatum lesions (Clarke, Robbins, & Roberts, 2008). In humans, patients with obsessive compulsive disorder displayed abnormal fronto-striatal functioning when tested on a reversal task. This decrease in striatal and OFC responsiveness was correlated with poor performance on a reversal task (Remijnse et al., 2006). It is possible a similar mechanism is at work in the study presented here. Repeated doses of amphetamine have affected the striatum so that fronto-striatal communication is no longer functioning properly, which would have upstream effects on the OFC itself. This, in turn, would also explain why attentional shifts were not impaired after repeated exposure.

Amphetamine's mechanism of action is similar to cocaine but it is not identical. In addition to its actions on neurotransmitter transporters, amphetamine also excites dopamine neurons by an indirect increase of glutamate activity in the striatum. In Supplementary Experiment 4, the glutamatergic neurons were inhibited using the NMDA antagonist MK-801. Under the influence of MK-801, rats were unable to complete the ASST. While on the drug, rats were faster to respond but significantly increased their error rate (see Figure 13). The increase in locomotor activity could be responsible for the decrease in latency (as seen in Figure 14) and

subsequently increasing the error rate; however it is possible that the decreases of dopamine caused by the blockage of NMDA receptors also played a role in the failure to perform the ASST. This suggests a role of glutamate activity in poor ASST performance as well as dopamine, serotonin and norepinephrine.

## Quinpirole

Repeated administration of quinpirole resulted in impairments to both stages of attentional shifts, IDS and EDS. This effect is consistent with data such as those of Haluk and Floresco (2009). Administration of quinpirole into the nucleus accumbens impaired set-shifting in rats. One potential theory for quinpirole's effect on performance is associated with its apparent connection to persistent behavior. Dysregulation in $D_2$ receptor binding in the striatum has been associated with obsessive compulsive disorder – a disorder in which persistent behavior is a key characteristic (Boulougouris, Castañe & Robbins, 2008). Repeated exposure to quinpirole has previously induced persistent behavior patterns in terms of continually choosing a direction on T-maze (Kontis et al., 2008) or displaying compulsive checking behavior (Szechtman, Sulis & Eliam, 1998). Boulougouris, Castañe and Robbins (2009) suggest this persistent behavior is due to sensitization to quinpirole which affects post-synaptic $D_2$ receptors predominantly. This receptor sensitization, in turn, suppresses areas such as the basal ganglia (Boulougouris, Castañe & Robbins, 2009). Quinpirole-induced obsessive behaviors can help explain the perseverative responding most often associated with poor ASST performance which is seen in the results of Experiment 3. Control level performance on the ASST requires rats to disengage from previous behaviors in order to learn or reverse the new rule at hand. Animals displaying a persistent behavior, will increase the perseverative errors made, and subsequently perform poorly on reversals and attentional shifts.

Similar to the effects of chronic amphetamine, the striatum could play a role in quinpirole's results. One study conducted by Kellendonk et al., (2006) observed the effect of overexpression of $D_2$ receptors in the striatum. They found that overactive $D_2$-receptor activity in the striatum results in impairments in behavioral flexibility. Even when the $D_2$-receptor overexpression is reversed, the impairment is maintained. In addition, chronic $D_2$-receptor activity increases the dopamine levels and $D_1$-receptor activation in the mPFC. Both of these alterations could result in deficits of behavioral flexibility. The chronic quinpirole could be stimulating the $D_2$ receptors of the striatum (since it was administered generally via i.p. injection) which then would increase dopamine levels and $D_1$ receptor acitivity in the mPFC – affecting the associative loop from striatum to PFC (Kellendonk et al., 2006). $D_1$ receptor activity in the PFC, like most of the dopamine receptors, has an inverted U-shaped effect on cognitive function. High doses of $D_1$ receptor agonists and/or antagonists both impair working memory (Seamans & Yang, 2004). Unfortunately, the results presented are unable to make a determination on $D_1$ receptors' potential role in behavioral flexibility impairments; therefore, this is one possible explanation offered.

Effect of $D_4$ Receptor Antagonist

The results also show blocking $D_4$ receptors via L-745,870 was able to attenuate the effects of the drugs on all the impairments found in the drug-only groups. $D_4$ receptors are highly localized to the frontal cortex (dorsolateral frontal, medial prefrontal, and entorhinal cortex), cortical regions surrounding the prefrontal cortex, the amygdala and hippocampus (Oak, Oldenhof & Van Tol, 2000; Tarazi, Kula & Baldessarini, 1997; Wędzony, Chocyk, Mackowiak, Fijal & Czyrak, 2000). This suggests that the $D_4$ receptors are working via the prefrontal cortex to improve the cognitive performance or temporarily mask the impairments caused by drugs of

abuse. Floresco et al. (2006) saw similar effects in drug naïve rats when given the same $D_4$ antagonist. When PFC $D_4$ receptors were blocked, rats decreased the number of trials required to reach a criterion level and also a reduction in the number of errors made. Conversely, a $D_4$ agonist had detrimental effects on shifts by increasing perseverative errors. Perseverative errors occur when the subject is unable to inhibit the previous rule that signaled reward. This is the case for both the reversals and attentional shifts. Floresco et al. (2006) suggest that increased $D_4$ receptor activity interfered with the inhibitory processes. By attenuating the effectiveness of $D_4$ receptors, the subject is better able to develop and maintain a new strategy for the new set of implicit rules.

One potential explanation the $D_4$'s effect on developing and maintain a new strategy in the attentional shift stages is its influence over the NMDA receptor. Floresco et al. (2006) have suggested that by either increasing or decreasing $D_4$ receptor activity, NMDA receptor activity responds inversely. Systemic injections of a $D_4$ agonist, PD168077, reduced the NMDA-receptor current in PFC pyramidal neurons. $D_4$ antagonists, however, blocked this reduction. PD 168077 even managed cause an internalization of NMDA receptors (Wang et al., 2003). Reducing the effectiveness of NMDA receptors can impair performance on attentional shifts predominately through increased perseverative responding (Stefani & Moghaddam, 2003). This is also observed in Supplementary Experiment 4. The percentage of correct choices made dropped significantly when the rats were tested on MK-801. This drug serves to block NMDA receptors, thus reducing their effectiveness. Chronic drug administration, namely cocaine and quinpirole where IDS and EDS impairments are observed, seemingly increased the number of trials needed to reach criterion whereas the L-745,870 treated rats were able to quickly reach criterion. Given the idea that blocking $D_4$ receptors subsequently enhances NMDA activity, the

damage caused by cocaine and quinpirole was attenuated by the increased NMDA activity. It is possible this mechanism of action is responsible for attentional shifts rather than reversal learning. Perseverative responding was no longer causing the animals to return to the previously rewarded stimuli. The combination of $D_4$ blockade and NMDA activity increased the behavioral flexibility even in those rats experiencing cocaine and quinpirole impairments.

The results also show that reducing $D_4$ activity was able to attenuate the effects of the amphetamine and cocaine on reversals. $D_4$ receptors are highly localized to the frontal cortex. In addition, $D_4$ receptors are found in superficial layers of pyramidal cells that receive sensory inputs, and less so in the layers associated with frontocortical modulation of striatal activity (Cocker, Le Foll, Rogers & Winstanley, 2013). This implies that $D_4$ antagonist's effect on reversal learning is most likely acting via the prefrontal cortex to attenuate the impairments caused by amphetamine and cocaine. One interesting, and somewhat surprising, effect was L-745,870 pretreatment induced a reversal impairment in the quinpirole group. In Reversal 1, the quinpirole-$D_4$ group required significantly more trials than the other three group. Reversal 2 displays an overall significance, though nothing appeared to be significant in the post-hoc tests. Piray (2011) suggests that greater $D_2$ receptor availability is correlated with faster learning in reversal stages because rats develop a higher learning rate for negative predicative error. This learning would decrease the perseverative errors in reversal stages (Piray, 2011). The quinpirole group presented here did not have reversal impairments, as would be expected based on Piray (2011); however, when $D_4$ receptors were blocked in this same group, impairments in the reversals were revealed. As previously stated, Kellendonk et al. (2006) suggested that increased $D_2$ activity also increases $D_1$ receptor activity in a compensatory manner. Supranormal $D_1$ receptor activation has also been reported to impair, not the acquisition of an associative cue but

the expression of the association (Lauzon, Bishop & Laviolette, 2009). Inability to apply newly learned associations can significantly impair reversal learning, which is possibly what is occurring in quinpirole rats tested on the $D_4$ antagonist.

Associative cues could be playing a major role in the presented studies, namely $D_4$ receptors' role in attribution of incentive salience to cues. A recent study by Cocker, Le Foll, Rogers and Winstanley (2013) observed the effect of $D_2$-like receptors on modulating reward expectancy. Their results show L-745,870 decreased the error rate of rats in a slot machine task. They suggest that blocking $D_4$ receptors dampens the salience placed on the cue previously associated with reward in a loss situation (Cocker et al., 2013). A similar effect could also be happening in the present study; however this idea was not tested empirically in the current study. Therefore, it is a speculation that the rats tested on the ASST task after L-745,870 administration may be placing less salience on cues previously associated with reward. If there is less salience for a specific cue, the rat may be more inclined to switch choices in the reversals or attentional shifts once a mistake has been made. The rats pretreated with L-745,870 may still have been experiencing the striatal damage that was caused by the repeated drug injections, but simultaneously exhibited a decrease in $D_4$ receptor activity during the test, which takes precedence. This suggests that $D_4$ antagonism can 'override' the salience of certain cues that the drug-treated rats were struggling to overcome. Cocker et al. (2013) reported that the $D_4$ antagonist was able to attenuate the impairments caused by quinpirole when both drugs were administered which supports the idea that $D_4$ receptor blockade can mask the damage seen in drug-exposed rats.

A decrease in cue salience could also explain the significant decrease in response latencies in the L-745,870 rats. It is possible that these rats are motivated by hunger to respond

for a reward but are spending less time deciding between the presented cues. The drug-only rats showed preservative responding, which implies a higher salience associated with the cue that had originally signaled reward.

It is important to note that the PFC is mediating subcortical structures in a top-down manner to improve performance via $D_4$ receptor blockade. Previous work by Laviolette, Lipski and Grace (2005) has shown that there is a small section of neurons in the mPFC that receives inputs from the basolateral amygdala (BLA). The connection between the PFC (the OFC in particular) and the BLA during encoding of cues is well documented (Schoenbaum, Chiba & Gallagher, 1998, 1999; Schoenbaum, Setlow, Saddoris & Gallagher, 2003). In a task better related to the ASST, namely visual discrimination reversal learning, data have shown that lesions to the BLA enhance performance on reversals and that animals with BLA lesions are more likely to correct their responses after negative feedback (Izquierdo et al., 2013). Izquierdo et al., (2013) suggest that lesions to the BLA increase the salience of negative feedback and subsequently allow the animal to use that feedback to make its next response. This evidence suggests that the BLA, in addition encoding cues, also plays a role in feedback regarding rewards.

Laviolette et al. (2005) have shown that the neurons in the mPFC that receive input from the BLA are $D_4$-receptor expressing neurons. They have done this by measuring the bursting rates of $D_4$-receptor neurons in the mPFC during fear conditioning. When given a $D_4$ antagonist, learning of an odor-footshock association was blocked. This was observed behaviorally by an increase in freezing time, and physiologically, by a lack of spikes in BLA neuronal activity (Laviolette, et al., 2005). Based on these data, $D_4$ receptor stimulation is necessary for the encoding of predictive cues.

It is also important to note these effects of $D_4$ receptors on cue encoding and salience appear to be dose dependent as evidenced by tests of working memory. Rats that have higher baseline performance on a task of working memory were impaired by higher doses of a $D_4$ antagonist and rats with lower baseline performance exhibited an inverted U-shape response to $D_4$ antagonist doses. Optimal performance was found between the doses of 0.05 mg/kg and 0.15 mg/kg (Zhang et al., 2004). Rats treated with drugs were operating at a lower baseline performance and therefore would seem to benefit the most from similar dose. The study presented uses a dose of 0.1 mg/kg, which aligns within the ideal range of doses. The 0.1 mg/kg dose was chosen based on the previous reports that this dose obtained optimal results without deleterious motor effects while being administered via i.p. injection rather than directly into the PFC. Also, since the study aimed to target $D_4$ receptors specifically, this dose is understood to have minimal interaction with other receptors of neurotransmitters which could interfere with the results (Zhang et al., 2004). While it was not tested with a range of doses, it is entirely possible that the results seen here would not be the same at higher or lower doses. A U-shaped response has occurred with low and high doses of L-745,870. Zhang et al. (2004) saw that in rats with good baseline performance, doses of .015 to .15 mg/kg saw no effect, while larger doses of 0.5 to 5 mg/kg actually caused impairments. In rats with poor baseline performance, the same lower doses improved performance. This U-shaped response, particularly in studies observing DA's effect on executive functioning, is seen frequently. Low doses of stimulants have been known to improve cognitive abilities, whereas high doses of the same stimulant can severely impair cognitive performance (Arnsten & Li, 2005). The same can be said for direct dopamine receptor stimulation. Dopamine $D_1$ agonists can impair working memory based on its dosing size (Sawaguchi & Goldman-Rakic, 1994; Zahrt et al., 1997). Based on these data, it is possible that

at higher or lower doses of the drug, even saline control rats could show impairments. Because of this dose-dependent effect, we acknowledge that the apparent ability of L-745,870 to improve reversal learning in this study may only be seen at this particular dose (0.1 mg/kg).

In addition to doses of the $D_4$-receptor antagonist, doses of the specific drugs may also be responsible for the effects seen in Experiments 1, 2 and 3. The doses that were used were chosen based on their ability to alter dopamine levels within the brain or because they had been used in behavioral tests similar to the ASST. It's possible that using higher or lower doses would result in different results. For example, a dosing regimen of 2 or 4 mg/kg of cocaine for 14 consecutive days resulted in reversal learning impairments (Jentsch, Olausson, De La Garza & Taylor, 2002). Perhaps at a dose lower than the 15 mg/kg used in Experiment 1, impairments may have only been seen in reversals and not attentional shifts. Since only one dose was administered for each group, the results presented in each experiment may only be found at those specific doses. These results may change when tested at higher and lower doses.

Another important caveat to note is the difference between acute and non-acute effects. For example, acute effects of amphetamine include increased anxiety-like behavior in correlation with a decrease in norepinephrine and serotonin availability in dentate gyrus and ventral hippocampus 20-24 hours post-injection (Barr & Forster, 2011; Barr, Renner & Forster, 2010). After 4 weeks of withdrawal, the anxiety-like behaviors remained despite a recovery to baseline serotonin availability; however, there was a significant decrease in cell proliferation (Barr, Renner & Forster, 2010). In cocaine users, acute effects include an increase in extracellular dopamine in the nucleus accumbens and decreased inhibition of responses (Fillmore, Rush & Hays, 2002; Kalivas & Duffy, 1990). Even in detoxification, reduced $D_2$ receptor availability associated with memory impairments has been reported (Volkow, Fowler & Wang, 2003). It

seems likely that the reversal learning impairments found are the result of the acute effects of drug administration, while studies such as Izquierdo et al., 2010; Kosheleff et al., 2012, Schoenbaum et al., 2004) are observing a long-term change caused by repeated drug administration.

In addition, it's possible what is seen in the results is the effect of withdrawal from the drug rather than the direct impact of the drug itself. Animals were tested 24 hours after last administration of a drug and some reports suggest that animals experience an anhedonic state during withdrawal from drugs (Markou & Koob, 1991). If animals are being tested during the anhedonic state, motivation to complete the task may be low and could result in poor performance overall. While this is possible, each drug tested resulted in impairments in different stages. Cocaine impaired both reversals and attentional shifts, amphetamine impaired reversals and quinpirole only affected attentional shifts. If amotivation and anhedonia were the causes, similar impairments would be expected in all three tests. In addition, arguments made in the discussion suggest a dysregulation of dopamine availability even 24 hours post-injection, and this has been supported by Weiss, Markou, Lorang and Koob (1992). A significant decrease in dopamine release was found 4-6 hours after cocaine self-administration and that decrease lasted for 12 hours. Even with the possible effects of withdrawal, dopaminergic dysregulation after drug use has been shown and therefore is still a viable explanation for the results of Experiments 1, 2 and 3.

The presented results help elucidate the beneficial role of $D_4$ receptors in the PFC. Blocking of $D_4$ receptors improved performance on the ASST even after chronic administration of drugs of abuse. While it still remains to be seen if $D_4$ receptor antagonism is simply masking

the cognitive deficits or reversing the damage, the results imply that $D_4$ receptors of the PFC should remain an area of interest for cognitive enhancers and anti-psychotics.

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