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Effects of Caffeine in Operant Learning and Locomotor Activity

Abstract

Caffeine is widely considered to be a reinforcer in humans, but this effect is difficult to measure in non-human animals. This study extended the understanding of the effects of caffeine on reinforcement and locomotor activity by testing rat animal models. A group of 24 adult male *Long Evans* rats was split into three groups, caffeine (15mg/kg), Scopolamine (spatial learning inhibitor) or a NaCl control. Each drug was administered 15 min prior to behavioural testing. The rats were given one habituation session to familiarize with eating the reinforcing agent, one baseline session before testing and on test day were monitored for the number of bar presses, vertical movements, horizontal movement and latency to first reinforcement across six 2 minute time bins. It was hypothesized that rats injected with caffeine would experience an increase in motivation for food reward, presenting as a greater number of bar presses compared to the control groups. Caffeine injected rats are expected to exhibit greater locomotor activity than control groups. Rats injected with caffeine had greater locomotor movement but had no positive reinforcement on bar presses and experienced a lower number of bar presses than the control group. Thus, the results of this study suggest caffeine had a negative reinforcement effect on operant learning mechanisms. Future studies should explore what could have caused this negative reinforcement interaction between caffeine and learning of the bar pressing reinforcement task.

Key words: Caffeine, Locomotor Activity, Reinforcement, Operant Learning, Motivation

Introduction

Caffeine is currently the most commonly taken psychoactive drug, used by a large percentage of the population mainly for its stimulant properties (Jarvis, 1993). Past studies have solidified our understanding of caffeine as a stimulant. It has been cited to increase general physiological arousal as measured by increased cortisol production, heart rate and respiration (Fisone, Borgvist & Usiello, 2004). In addition to its stimulant properties, caffeine is often used as a reinforcing agent in human studies (Griffths et al., 1988). While caffeine's reinforcement effects have been difficult to replicate in non-human subjects (Sheppard et al., 2012) its effects on locomotor activity have been found in both human and non-human experiments.

Antoniou et al.'s 1998 study tested the effects of a number of drugs including caffeine, amphetamines and cocaine on a number of paradigms to test for locomotor activity. The researchers demonstrated that low and medium doses of caffeine produced higher locomotor activity in rat subjects compared to control subjects who did not receive the drug. This discovery is important for understanding how caffeine influences behaviour. To further understand the mechanisms of caffeine use, recent studies have begun to explore how caffeine interacts with other drugs.

In combination with cocaine, caffeine was found to increase excitation of subjects further increasing locomotor activity; it also increased the reinforcement effects of cocaine (Mehta, Jain, & Billie, 2004 and Kunin et al., 2000). Caffeine in combination with both amphetamines and cocaine has been shown to significantly increase level of self-drug administration in both non-human primates and rats (Comer and Carroll, 1996). Current research has furthered the study of caffeine on operant conditioning using reinforcing agents other than drugs. For example in 1993,

Buffalo, Gillam, Allen and Paule, found that rhesus monkeys given caffeine showed behavioural alterations that caused them to improve at both learning and reinforcement tasks.

According to the study conducted by Fernstrom and Fernstrom's (1984) the mechanism, of caffeine altering reinforcement, may be caused by changes in the release, reuptake and use of dopamine in response to caffeine consumption. Dopamine is a neurotransmitter found in various different brain regions, including the nucleus accumbens. It is a key player in the reward pathways of the brain and in understanding the neural mechanism of why addiction and self-administration of drugs, or non-drug reinforcers, work. The results of an investigation carried out by Solina et al. in 2002 showed that caffeine facilitated dopaminergic neurotransmission by stimulating dopamine release and potentiating dopamine receptor stimulation inside of the nucleus accumbens. This resulted in greater activation of the reward pathway of the brain when reinforcement in either drug or non-drug forms were used.

To understand how caffeine alters reinforcement and operant learning on a behavioural level, researchers have measured operant learning using novel tasks. One example implemented by Bergen, Spratt & Messier (1997) successfully measured operant learning using the bar pressing task in the rat animal model. Other studies looking at the relationship between operant learning and caffeine have focused on motivations for food reward in subjects (Sheppard et al., 2012). These studies have not yet looked at how caffeine affects operant learning in food reinforcement tasks. Sheppard et al.'s (2012) study demonstrated that caffeine indirectly increased in the reinforcing effects of lever presses, leading to food reward, which in turn led to more lever presses. Although this study clearly showed that caffeine causes increased motivation for operant learning. The extent to which it acts as a reinforcer was not revealed.

Study by Smith, Hunter and Bennett (1994), demonstrated the use of Scopolamine as a successful memory inhibitor in the Morris Water Maze task and therefore a successful control group. It is expected that this effect would carry over to the bar pressing task in which rats must first learn the connection between bar press and food reward. Scopolamine is expected to block the learning of rats, decreasing performance levels in any bar-pressing food reward reinforcement tasks. A reinforcement schedule using a bar pressing task and a food reward will therefore be used to test operant learning of caffeine injected and control.

The current study plans to extend the study of caffeine and how it interacts with locomotor activity of subjects by testing on medium doses of caffeine and exploring how locomotor activity may change over time as caffeine is metabolized. Furthermore, this study intends to explore the relationship of caffeine and reinforcement learning therefore extending the understanding of how caffeine affects the rat animal model. It is hypothesized that rats injected with caffeine will experience an increase in motivation for food reward, presenting as a greater number of bar presses compared to the control groups. Caffeine injected rats are expected to exhibit greater locomotor activity than control groups.

Method

Participants

Subjects were male *Long Evans* rats (325-375g) taken from Charles River, Quebec. The animals were paired and housed in polypropylene cages in a colony room at 21 ± 1 °C. Rats were kept in a 12:12 light dark cycle that began at 0700h and habituated to a food deprivation schedule while maintained at 90% of pre-deprivation body weight. Rats were handled, and tested according to the guidelines that were set by the Canadian Council on Animal Care (CCAC).

Apparatus and Materials

Subjects were placed in a operant testing chamber(43 cm X 35 cm X 30 cm) made up of plywood with a single clear Plexiglas front panel with a retractable lever near a food pellet dispenser that was designed to provide reinforcement for every bar press(FR-1). Locomotion was measured by two observers and a chronometer was used to time latency to first response.

Drugs

Each of the subjects were injected with either 15 mg/kg caffeine (Lot# SLBH1002V; Sigma, St. Louis, MO) dissolved in 0.9% saline, 1 mg/kg of scopolamine hydrobromide (Sigma, St. Louis, MO) dissolved in 0.9% saline or a control injection of 0.9% saline. Volume of injection was 1 ml/kg; all injections were given intraperitoneal (i.p.).

Procedure

Rats first received a habituation session in which they were placed inside the testing chamber to familiarize themselves with eating the reinforcing agent (sweetened food pellets-Test Diet purified rodent table 5TUL). One week before testing day rats received two 10 min shaping sessions in the testing chamber, in which reinforcement tablets were given to cause rats to learn that pressing the bar would release food. This was done by first reinforcing movements towards the bar and eventually only reinforcing touching the bar until the rat eventually learned the press the bar. At the end of the two 10 min sessions rats were expected to have learned that pressing the bar would allow them to release food themselves. Three days before test day rats received one baseline test session in which they were place in the testing chamber for 12 min and the number of bar presses were monitored.

Rats were injected i.p. with one of the three drugs 15 minutes prior to placement in the operant test chamber. Rats were assessed for 12 minutes split into 6 time bins. Locomotion was assessed by looking at horizontal and vertical movements. A horizontal movement was defined

as any movement that resulted in two paws crossing any quadrant line in any direction. A vertical movement was defined as any movement which resulted in the rats paws being off the ground and its nose passing above the horizontal line inside the open-field apparatus. Number of bar presses, rate of response and latency to first response were all measured during testing to understand how the different drugs may affect operant learning of rats. A comparison between pre-drug manipulation testing and post-drug manipulation testing of total number of bar presses was also done. All instances of any movement, bar press or rate of response were recorded by two observers and a third observer timed the latency to first response.

Treatment of Data

To test for inter reliability of both observers for horizontal and vertical movement a Pearson correlation test was used. The correlation was considered significant if $r > 0.5$. Horizontal movement, vertical movement and response rate were analyzed using a 3 x 6 split-plot ANOVA. The ANOVA was done between the different groups at 3 levels (caffeine, scopolamine and NaCl control) within 2 minute time bins at 6 levels. Latency for first response was analyzed by comparing the average of each between subjects group (caffeine, scopolamine and NaCl control) using a between subjects one way ANOVA. A 3 x 2 split-plot ANOVA was used for comparing the total number of responses for pre and post drug manipulations between groups at 3 levels (caffeine, scopolamine and NaCl control) and within 2 levels of total responses (pre and post drug manipulation). A criterion of $p < .05$ was used for all ANOVA tests.

Results

Horizontal Movement

A high inter-rater reliability factor was found between observers, therefore justifying the use of only one set of data for analysis, $r(21) = 0.94$. The results of the 3 x 6 split-plot ANOVA

showed a significant main effect of drug group on horizontal movement ($F(2, 21) = 48.50, p < .001$). Specifically, a significant difference was observed between Caffeine and Scopolamine drug groups for horizontal movement. A significant difference between Caffeine and the control NaCl group was also observed for horizontal movement. Scopolamine and the control NaCl group did not significantly differ for horizontal movements (see Figure 1). A significant main effect of time bins on horizontal movement was found ($F(5, 21) = 16.59, p < .001$). Generally a significant decrease was seen across time bins (see Figure 1). A significant interaction effect between drug group and time bins was found, ($F(10, 105) = 2.85, p < .01$). Both Caffeine and control NaCl groups showed a decrease over time bins while, Scopolamine showed very little change over the six time bins. Over six time bins, Caffeine and NaCl control groups experienced a large decrease in horizontal movements while Scopolamine stayed consistent. Caffeine experienced the greatest decrease in horizontal movement across the 6 time bins and also demonstrated the greatest number of horizontal movements.

Vertical Movement

A high inter-rater reliability factor was found between observers, therefore justifying the use of only one set of data for analysis, $r(21) = 0.90$. The results of the 3 x 6 split-plot ANOVA showed a significant main effect of drug group on vertical movement ($F(2, 21) = 5.53, p < .001$). The main effect of drug groups was likely driven by the significantly greater vertical movement found in the caffeine group in comparison to Scopolamine and control groups (see Figure 2). A significant main effect of time bins on vertical movements was found ($F(5, 21) = 16.49, p < .001$). The significant main effect of time bins was likely driven by the decrease in vertical movements across the 6 time bins (See figure 2). A significant interaction effect was found for drug groups within time bins ($F(10, 105) = 3.18, p < .01$). Specifically, caffeine and

NaCl control groups both showed a significant decrease in vertical movement in the first time bin which then leveled out into a steady number of vertical movements, while Scopolamine showed a non-significant change in vertical movement across the 6 time bins (see Figure 2). Across all 6 time bins Caffeine was observed to significantly decrease while Scopolamine showed no change in vertical movement. The Caffeine group demonstrated the greatest number of vertical movements throughout most of the observation period.

Response Rate

The data analysis using a 3 x 6 split-plot ANOVA revealed a significant main effect of drug group on response rate ($F(2, 21) = 93.84, p < .001$). The Caffeine group showed a significantly lower response rate when compared to the control NaCl. The Caffeine group also showed a significant greater response rate than the Scopolamine group (See Figure 3). A significant main effect of time bins on response rate was found ($F(5, 21) = 16.49, p < .001$). An increase from time bins 1 to 2 followed by a steady decrease of response rate from time bins 2-5 and a flattening out from time bins 5-6 was the general trend found in figure 3. A significant interaction effect was found for drug groups within time bins for response rates ($F(10, 105) = 3.29, p < .01$). Scopolamine injected rats had no significant change from time bins 1-2 followed by a decrease from time bins 2-3 then a flattening out with no change from time bins 3-6 the trend of caffeine and NaCl control group within time bins was significantly different. Specifically, Caffeine and NaCl control groups declined significantly from time bins 2-5 while the Scopolamine group showed no significant change during this time period. In summary, the greatest response rate was found in control NaCl groups. The Caffeine group demonstrated a significantly lower response rate than the control group. Both the control and caffeine groups

showed an increase of response rate at the beginning of the experiment followed by a steady decrease in response rate until the 6th time bin.

Latency to first response

A one way between subjects ANOVA demonstrated a significant difference between drug groups for latency to first response ($F(2, 21) = 5.07, p = .02$). Caffeine and control group did not significantly differ for latency to first response. Both Caffeine and control groups demonstrated significantly shorter latencies to first response when compared to the scopolamine drug group (see Figure 4).

Total number of pre vs. post drug manipulation responses

The 3 x 2 split-plot ANOVA analysis revealed a significant main effect of drug manipulation on total number of responses ($F(2, 21) = 50.85, p < .001$). Specifically, the Scopolamine group was observed to have a significantly different number of responses than either the control NaCl or Caffeine group. The caffeine group and Control groups did not significantly differ on their total number of responses (see Figure 5) The pre drug manipulation had a significantly greater number of total responses than the post drug manipulation ($F(1, 2) = 156.93, p < .001$). A significant interaction between drug group within pre and post drug manipulation was observed ($F(2, 21) = 61.38, p < .001$). To be exact, the Scopolamine group significantly differed in pre and post drug manipulation for total number of responses while, Caffeine and control groups did not differ significantly between pre and post drug manipulations (see Figure 5). The Scopolamine drug group demonstrated an effect of drug manipulation on total number of responses for pre and post drug manipulation. In post drug manipulations Scopolamine demonstrated a significantly lower number of responses while the Caffeine group showed no significant difference.

Discussion

It was hypothesized that caffeine injected rats were expected to experience greater locomotor activity than control groups. Rats injected with caffeine were also expected to experience an increase in motivation for food reward and therefore display a greater number of bar presses than the control groups.

The locomotor activity of caffeine-injected rats supported the hypothesis of this study. The caffeine group was found to have a greater number of both vertical and horizontal movements throughout the experiment than either the control or the Scopolamine group. This adds support to previous findings that specific doses of caffeine increase locomotor activity (Bedingfield, King and Holloway, 1998). It is likely that a dose greater than the 15mg/kg used in this study may lead to a decrease in locomotor activity. In Antoniou et al.'s 1998 study it was found that a dose of 20 mg/kg would have an opposite effect to the group given 5 or 10 mg/kg of caffeine. These results are contrary to results found in 1982 by Carney in which 20 mg/kg of caffeine was found to be a peak concentration for locomotor activity.

When comparing the amount of reinforcement bar presses between the pre-drug manipulation data and the post-drug manipulation data no significant difference was detected for the caffeine group. This is supported by results of latency to first bar press which did not differ significantly between the caffeine and control groups. This likely means caffeine did not help the subject to learn the relationship between bar press and reward any faster than the saline control. Furthermore, the control group was found to have significantly more bar presses per time bin than the caffeine group. It is possible that caffeine had a negative effect on reinforcement of food reward. This indicates that the results do not support the hypothesis, and that caffeine did not have a positive effect on reinforcement over the control and Scopolamine injected group.

The negative reinforcement of bar presses observed with the caffeine group may have been caused by a number of factors. Two human caffeine ingestion studies (Jessen et al., 2004 and Belza, Toubro & Astrup, 2009) found that caffeine itself was able to suppress appetite and that it could increase appetite suppression caused by other drugs. This lowered appetite would lead to decreases in the need for food reinforcement. This would imply that the experiment is no longer viable for measurement of operant learning, since the reward is no longer desirable enough to the subject to create a reliable conditioned stimulus reaction.

Another theory of why caffeine may have caused negative reinforcement is the dosage used in the current study. A dose of 15mg/kg may have been too high, leading rats to lose motivation to seek food rewards. This occurred in Buffalo et al.'s, (1993) study in which rhesus monkeys injected with higher doses of caffeine showed less conditioned responses to the conditioned stimulus due to lack of motivation leading to a learning deficit. This lack of motivation may be become present in rats injected with 15mg/kg of caffeine as well. To solidify which theory is driving the effect or if both begin to play a part at 15 mg/kg further studies should replicate this study while replacing control groups with various doses of caffeine. These future studies would make it clear whether doses of caffeine may play a part in how caffeine alters operant learning, motivation and general reinforcement of non-drugs.

The current study looked mainly at the effects of caffeine on operant learning in comparison to Scopolamine and a saline control group as well as the difference in locomotor movement when subjects were injected 15 minutes prior to the experiment. However future studies should study how different schedules of administration may change its effects on learning. Previous findings in studies observing the effects of caffeine on spatial learning paradigms have indeed found variation in results when testing different schedules of

administration (Angelucci, Cesario, Hiroi, Rosalen & Cunha, 2002). This warrants future research into whether variations in schedules of administration of caffeine strengthen or weaken the reinforcing effect of non-drug rewards. Further applying principles in this study to human studies would also allow for greater generalization of results, namely the effects of caffeine on conditioned responses to non-drugs. Further studies are warranted to test the theories of negative reinforcement caused by decreases in motivation. These studies should attempt to replicate the current study while using different non-drug reward stimuli that are not food. To further understand how caffeine may be an appetite suppressor more research should be conducted specifically looking at caffeine's effects on appetite. Future studies should also explore which dose of caffeine produces the peak locomotor activity and which doses would begin to lead to a decrease in locomotor activity. This research can then be used to create a better procedure for testing caffeine's effects on operant learning.

In conclusion, the first hypothesis of this study was supported. Caffeine caused increases in locomotor activity as was demonstrated by significant increases of horizontal and vertical movement over both control groups. The second hypothesis was not supported in this study. In fact, caffeine was found to be a negative reinforcer of the bar pressing task and demonstrated a significantly smaller number of bar presses in compared to the NaCl control group. This may have been caused by caffeine causing a lack of motivation or suppressing appetite. Further research needs to be done on both theories so as to understand whether food as a reward is viable or may be causing a negative reinforcement. This study adds to the body of literature stating that caffeine effects locomotor activity. The results of the current study warrants more research into how caffeine interacts with operant learning.

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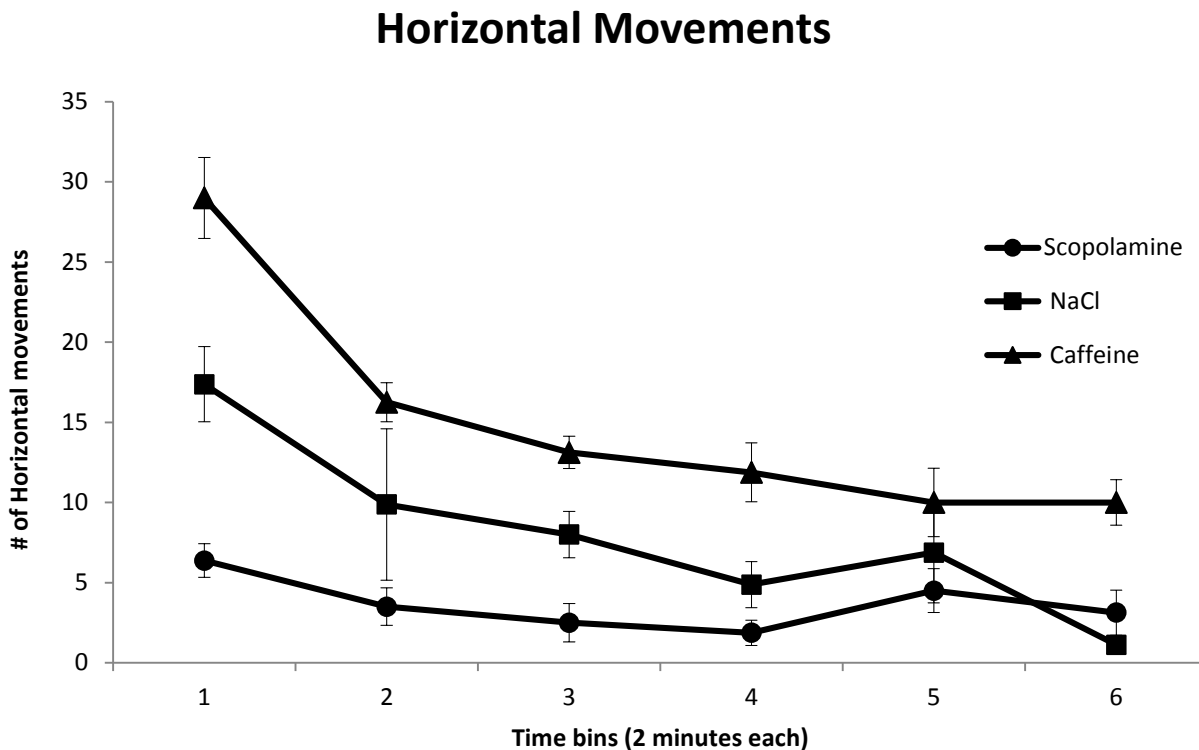


Figure 1. The mean number of horizontal movements of subjects ($n=24$) across 6 two minutes time bins. Mean horizontal movements \pm SE of male *Long Evans* rats observed 15 minutes after intraperitoneal injection of caffeine (15mg/kg), NaCl control or scopolamine (1mg/kg) as a function of six, 2 minute time bins. The caffeine group had a significantly greater number of horizontal movement than either control or scopolamine groups. A significant difference was observed at the first time bin, in general a decrease was seen across time bins. An interaction effect between caffeine and scopolamine within the 6 time bins was observed. Caffeine and NaCl both demonstrated a significant decrease in horizontal movements across 6 time bins. Error bars represent standard error of means.

Vertical Movements

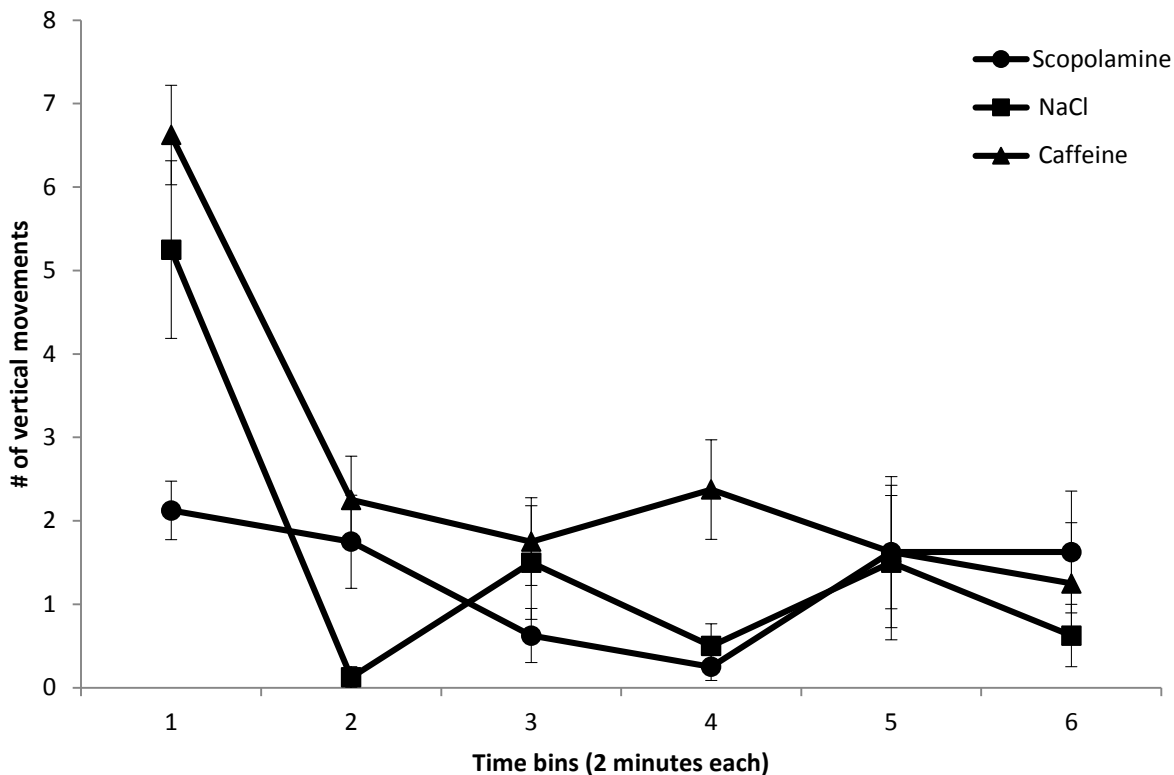


Figure 2. Vertical Movement across six time bins for each subject ($n=24$). Mean vertical movements \pm SE of male *Long Evans* rats observed 15 minutes after intraperitoneal injection of caffeine (15mg/kg), NaCl control or scopolamine (1mg/kg) as a function of six, 2 minute time bins. A significant main effect of drug group was likely driven by the significantly greater number of vertical movements in the caffeine group compared to the Scopolamine and control groups. Both NaCl and Caffeine groups significantly decreased across time bins. While across all 6 time bins Scopolamine had a non-significant change in the number of vertical movements. Error bars represent standard error of means.

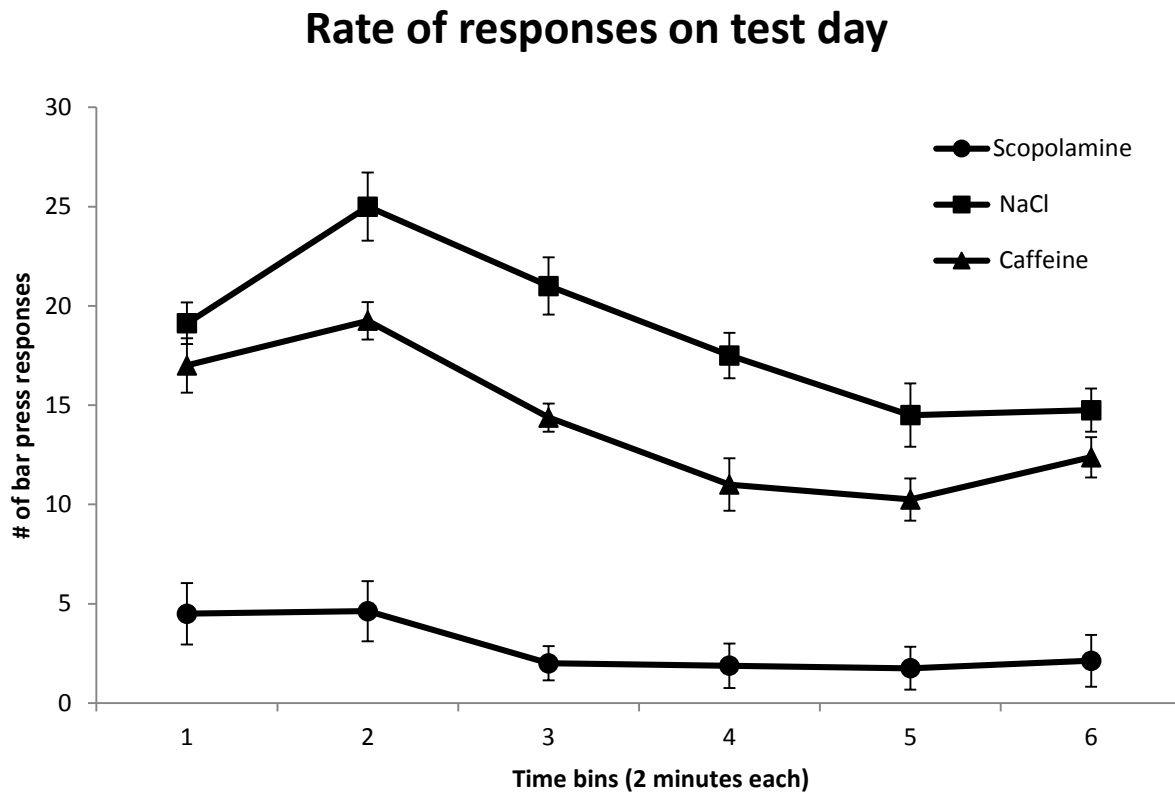


Figure 3. The response rate after drug manipulation across six 2 minute time bins for subjects ($n=24$). The mean number of bar presses \pm SE of male *Long Evans* rats observed 15 minutes after intraperitoneal injection of caffeine (15mg/kg), NaCl control or scopolamine (1mg/kg) as a function of six, 2 minute time bins. The saline group drove the main effect of drug group as it was found to have a significantly greater response rate than both caffeine and Scopolamine groups. The control and Caffeine groups both started at a high number of test day responses and experienced an increase in number of test day of responses per bin that was followed by a decrease until the end of the experiment this was significantly greater than the Scopolamine group which had a low amount of test day responses per bin, throughout the time bins with no significant change within time bins. Error bars represent standard error of means.

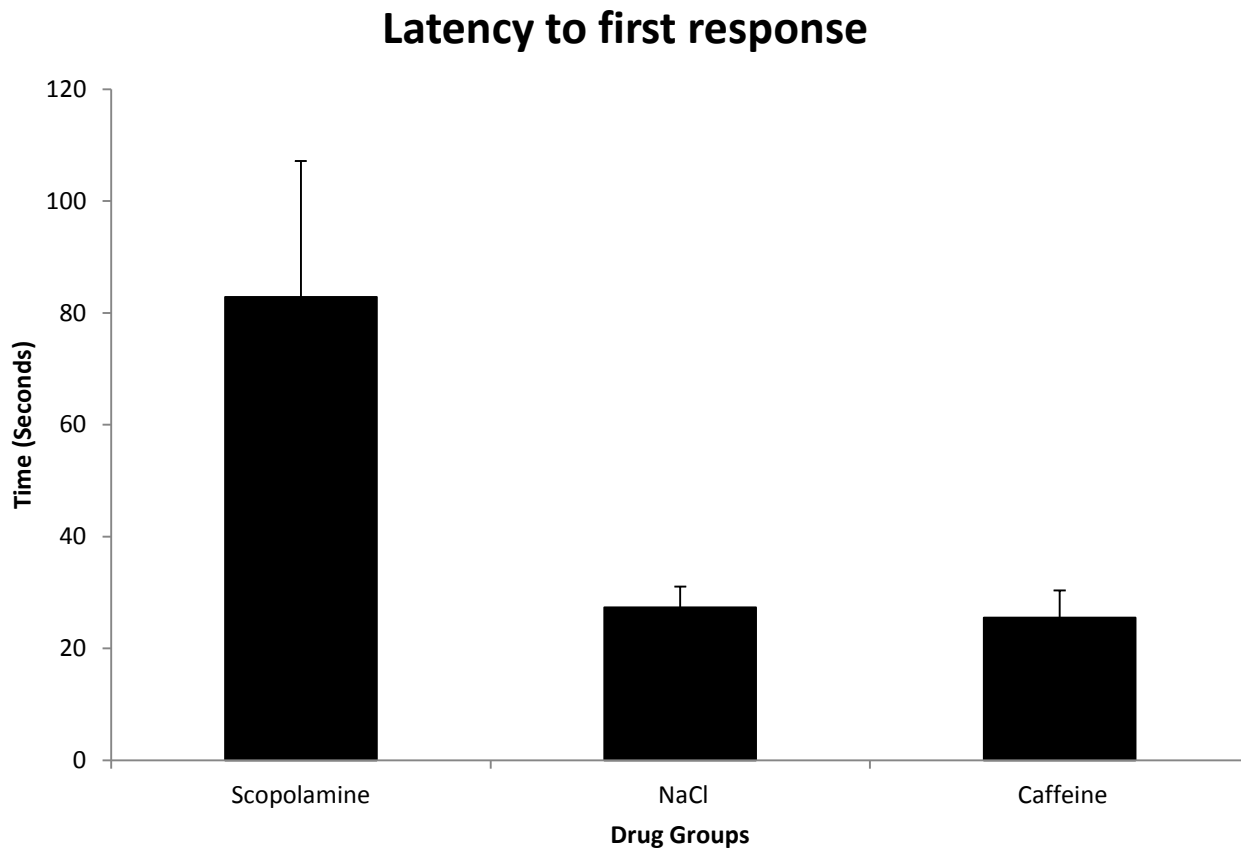


Figure 4. Latency to first response between three drug groups for subjects (n=24). Scopolamine, NaCl and Caffeine groups were injected intraperitoneal 15 minutes prior to operant behavior observation testing bar presses which lead to food reward. Latency to first bar press response was measured in seconds using a chronograph and compared between Scopolamine, NaCl and caffeine drug groups. The Scopolamine injected group showed a significantly longer latency to first response than Caffeine and NaCl drug groups. Caffeine and NaCl drug groups did not significantly differ for the latency time to first bar press. Error bars represent standard error of means.

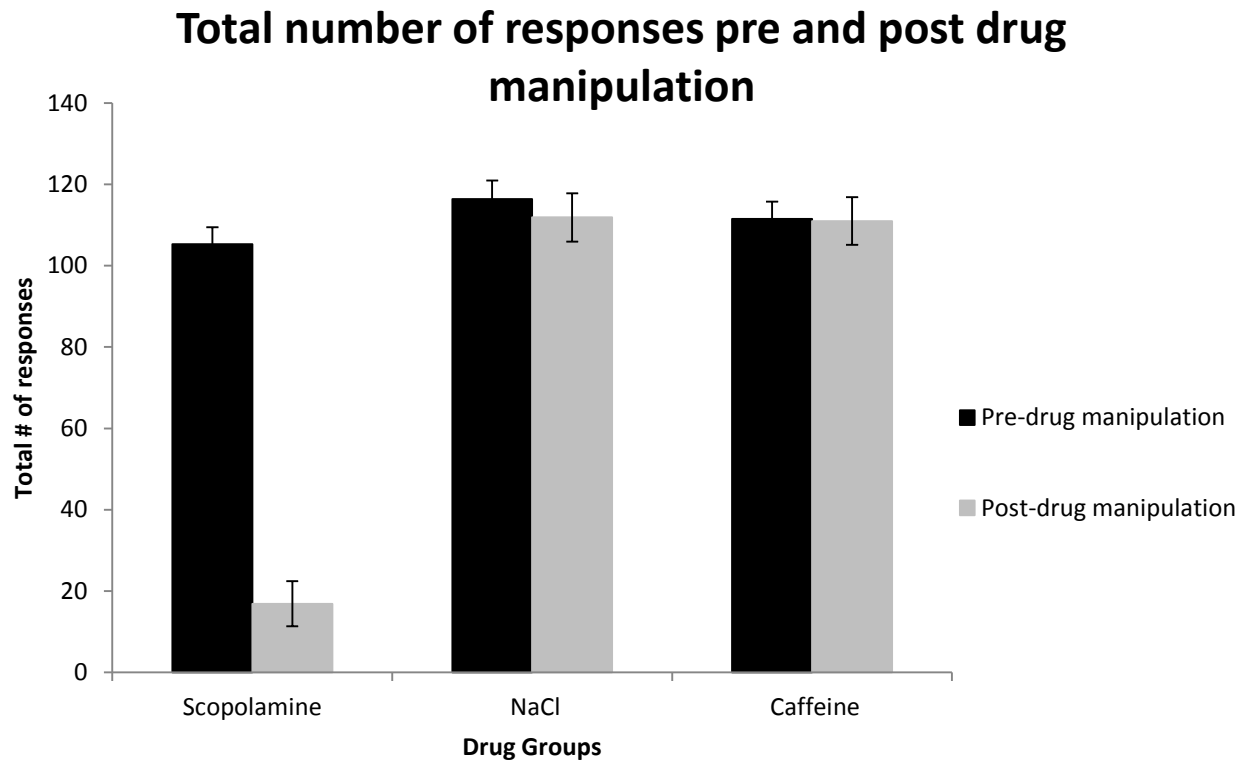


Figure 5. The total number of responses for pre-drug manipulation plotted against the total number of responses after a drug manipulation \pm SE of male *Long Evans* rats observed 15 minutes after intraperitoneal injection of caffeine (15mg/kg), NaCl control or scopolamine (1mg/kg). The Scopolamine group showed a significant decrease for the total number of responses from pre to post drug manipulation. The total number of responses for post-drug manipulation for the Scopolamine group was significantly lower than either NaCl or Caffeine groups. There was no significant difference between the three drug groups for pre-drug manipulation. No significant difference was observed between NaCl and Caffeine groups for the total number of response for post-drug responses. No significant difference was shown for the caffeine group from pre-drug manipulation to post-drug manipulation for total number of responses. Error bars represent standard error of means.