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Effects of Caffeine on Memory in Rats

Cisse Nakeyar

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Abstract

Caffeine is typically used to counter the effects of fatigue by enhancing performance for cognitive tasks, it is also known to suppress appetite. The current study is conducted to determine if caffeine would have an effect on memory. Twenty-four male Long Evans rats (375-425g) were used, they were randomly assigned to one of three groups: saline control (0.9% saline), caffeine low dose (15mg/kg), and caffeine high dose (50mg/kg). Testing was conducted in a Skinner box with a retractable lever that dispensed food when pressed. Rats were trained for five consecutive days for 10-15 min sessions. Then, two days of baseline testing was conducted followed by the treatment of each group on the test day. Results indicated that the low dose group had a significantly lower response rate compared to the control group. Similarly, the high dose group also had a lower response rate compared to the control. And, the low dose group had a higher response rate compared to the high dose group. Overall caffeine decreased the response rate but increased locomotor activity in rats. The present study does not support the hypothesis that caffeine increases bar pressing and improves retrieval memory for a food reward, likely by suppressing appetite.

Effects of Caffeine on Memory in Rats

Caffeine is a commonly used psychoactive drug to counter the effects of fatigue and to increase physical activity (Klaassen et al., 2013). It also has the potential to alleviate symptoms of Alzheimer's disease, Parkinson's disease, and obesity (Pierard, Ali, Henkous, Decorte, & Béracochéa, 2015; Collins et al., 2010). There is much literature on the effects of caffeine on memory, the current study will expand on past studies by evaluating if caffeine has any effect on recall memory in rats.

Pierard et al. (2015) investigated the effects of caffeine on rat's memory recall. They placed rats in an apparatus that had four holes, the contextual serial dimension (CSD) had two trials (D1 and D2) and had different coloured floors with food placed in different holes for each trial. The serial spatial dimension (SSD) had the identically coloured floors but the food was placed in different holes for each trial. The rats were in one of three groups: control (distilled water), caffeine low dose (16mg/kg), and caffeine high dose (32mg/kg). Pierard et al. (2015) found that caffeine improved retrieval memory in both CSD and SSD, however there was a dose dependent effect. The low dose group showed improved contextual recall for D1 in CSD only while the high dose group showed improved spatial recall for D2 in both CSD and SSD.

Previous research found that the hippocampus was involved in the recall of D1, indicating a contextual process, and that the prefrontal cortex was involved in the recall of D2, indicating serial order (Pierard et al., 2015). The study conducted by Pierard et al. (2015) was not the only one to indicate that the hippocampus and prefrontal cortex are involved in the retrieval of memory. Banich and Compton (2010) mention that memory had three processes: encoding, consolidating, and retrieving, and that the hippocampus and prefrontal cortex are both involved

in retrieving, thus suggesting that caffeine could improve recall if it does act on those brain regions.

Nehlig (2010) reviewed a list of studies to determine if caffeine is a cognitive enhancer. The study found that caffeine reduced reaction time and had an effect on mood, and that caffeine improved incidental learning rather than intentional learning. Considering the rats in the present study will be learning passively, caffeine should improve bar pressing (Nehlig, 2010).

In the Pierard et al. (2015) study the food was placed in one of four holes in the apparatus which simply required the rat to find it and remember where it was. The present study will be using a skinner box which requires the rat to learn that bar pressing provides food. But, would rats prefer to bar press for food or just free load? Jensen (1963) sought to demonstrate that bar pressing has some sort of intrinsic appeal. The results indicated that rats prefer bar pressing over eating from a cup which required no effort. Therefore, rats do have some form of intrinsic appeal for bar pressing for food. As well, Lau and Falk (1993) conducted a study to determine which administration method would be the most effective at delivering caffeine. They found that intraperitoneal (IP) injection was the most effective method, as such the current study will deliver caffeine and control via IP.

Past studies have demonstrated that caffeine improved recall memory, and that rats preferred bar pressing for food over receiving food without effort. The current study expands on previous findings by observing the effects of caffeine on recall memory during the bar pressing task. The dependent variables in the study are the number of bar presses, latency to bar press, a pre-treatment and post-treatment comparison, and horizontal and vertical movement (locomotion). The independent variables in this study are the conditions (NaCl control, low dose

caffeine, high dose caffeine). Based on the previous literature it is expected that caffeine will increase bar pressing behaviour in a dose dependent manner.

Method

Subjects

Subjects used for the experiment were 24 male Long Evans rats (Charles River, Quebec) weighing about 375-425g at the start of the experiment. The rats were housed in pairs in polypropylene cages and placed in a colony room with the temperature set at 21°C ±1. Rats were habituated to a food deprivation schedule and were maintained at 90% of pre-deprivation body weight but water was available ad libitum. The rats were on a 12 hour light-dark cycle beginning at 0700 hrs. For the experiment, the rats were placed in one of three groups; the NaCl injected group (control), low dose caffeine group and the high dose caffeine group (n=8/group). Animals were handled and tested in accordance with the guidelines set out by the Canadian Council on Animal Care (CCAC).

Drugs

There were three groups: NaCl injected controls (.09% saline), 15mg/kg of caffeine dissolved in 0.9% saline or 50mg/kg of caffeine (Lot# SLBH1002V; Sigma, St. Louis, MO) dissolved in 0.9% saline, each were administered intraperitoneal at a volume of 1ml/kg 15 min prior to testing.

Materials

In the study, an open field apparatus was used. The apparatus was 43 cm X 35 cm X 30 cm, it was made of plywood with a Plexiglas front panel for observation, which had a retractable lever beside food pellet dispenser designed to provide reinforcement for every bar press at a fixed ratio of one bar press for one food pellet (FR-1, Test Diet purified rodent table 5TUL). The amount of bar presses were measured (responses), as well as latency, the amount of time it took

the rat to begin bar pressing at the start of every 12 min trial. The floor of the apparatus was divided into six gridlines and horizontal movement was said to have occurred anytime a rat crossed one of the gridlines with any two paws. Vertical movement was said to have occurred anytime a rat had his two front paws off the ground and his snout crossed the vertical line.

Training

Rats received habituation session where they were placed in the apparatus to familiarize with eating reinforced food pellets (Test Diet purified rodent table 5TUL). Each rats was trained one week prior to testing, every day for five days with 10-15 min training sessions anytime between 0900 hrs to 1600 hrs. The rats began the experiment with no previous experience with bar pressing. Observers had a remote that was connected to the apparatus to provide reinforcement to rats in order to train them to bar press. The rats first learned that being close to the bar earned them food, then smelling the bar and finally they learned that pressing the bar resulted in them receiving food. Following training, rats conducted two days of baseline testing in the apparatus for 12 min and the total number of bar presses were monitored. The average total of bar press between the two days were used as the rats baseline bar press prior to treatment with their respective drug group.

Testing

On the test day rats received their respective drug via IP injection 15 min prior to testing. Observers did not know which drug a particular rat received. The rats were placed in the apparatus and observed for 12 min each. The 12 min trials had six different time bins. During those times the total number of bar presses, the rate of response, latency to first response, and locomotor activity (horizontal and vertical movement) were measured.

Data Analysis

The inter-rater reliability was measured using a Pearson correlation. A split-plot analysis of variance (ANOVA) was conducted to determine if the groups and time bins were different for response rate, pre-treatment and post treatment response rate, horizontal movement, and vertical movement. This compared the between-subjects factor (NaCl control, caffeine low dose, caffeine high dose) with the within-subjects factor (time at six levels). The pre-test and post-test comparison, however, had the same between-subjects factor (NaCl control, caffeine low dose, caffeine high dose) but had the within-subjects factor as the pre-test and post-test trials. The latency to response was analysed using a one-way ANOVA. The independent variable, the conditions, had three levels (NaCl control, caffeine low dose, caffeine high dose), and the dependent variable was the time it took each group to press the bar.

Results

Inter-rater reliability

The inter-rater reliability check was conducted using a Pearson correlation. It was found that the horizontal movement between the two raters was strongly correlated, $r(142) = 0.93$, indicating that data from one rater was used for the horizontal movement analysis. Also, the Pearson correlation found that vertical movement between the two raters was strongly correlated, $r(142) = 0.91$, as such data from one rater was used for the following analysis of vertical movement.

Pre-Treatment vs Post-Treatment Response

A main effect for group was obtained, $F(2, 21) = 4.24$, $p = .028$. The NaCl control group had the highest response rates followed by the low dose caffeine group and then high dose caffeine group had the lowest response rate (see Figure 1).

There was no main effect for day, $F(1, 21) = 0.12$, $p = 0.733$. There was no difference in the rate of response across the different days (see Figure 1).

But, an interaction effect was found, $F(2, 21) = 8.73$, $p = .002$. The high dose caffeine group had significantly more responses in the pre-treatment day compared to the post-treatment day. The NaCl control also had significantly more responses in the pre-treatment day compared to the post-treatment day. But, the low dose caffeine group was not significantly different between the pre-treatment and post-treatment day (see Figure 1).

Response Rate

A main effect for group was found, $F(2, 21) = 12.20$, $p < .001$. The NaCl control had the highest response rate, this was noticeable at time two, four and six. The low dose caffeine group had higher response rates compared to the high dose group but not the NaCl control, this is noticeable at time one and six. The lowest response rate was achieved by the high dose caffeine group (see Figure 2).

There was no main effect for time, $F(5, 105) = 1.12$, $p = 0.357$. Response rates were not significantly different across time, however, the trend seems to be that the NaCl group had more responses, the low dose caffeine had the second highest response rate and the high dose group had the lowest response rate (see Figure 2).

Similarly, no interaction effect was observed, $F(10, 105) = 1.47$, $p = 0.160$. There was no significant difference among groups across time in the response rate. The trend seemed to be that as time elapsed the low dose and high dose caffeine groups response rate decreased while the NaCl group increased (see Figure 2).

Latency to First Response

A main effect for group was obtained, $F(2, 21) = 3.53$, $p = 0.048$. The high dose caffeine had the highest latency to response, the low dose caffeine group and the NaCl control were not significantly different (see Figure 3).

Horizontal Movement

A main effect for group was obtained, $F(2, 21) = 13.35$, $p < .001$. The low dose and high dose caffeine groups displayed more horizontal movement than the NaCl control group. However, no significant difference was found between the low dose and high dose caffeine groups for horizontal movement (see Figure 4).

Similarly, a main effect for time was obtained, $F(5, 105) = 13.42$, $p < .001$. The amount of horizontal movement decreased from time one to time two, and increased from time five to time six (see Figure 4).

Lastly, a significant interaction was found, $F(10, 105) = 1.96$, $p = 0.046$. The NaCl control group maintained the lowest amount of horizontal movement across all times, while low dose and high dose groups were significantly different at time one where the high dose caffeine group had less horizontal movement than the low dose caffeine group. Then, from time two to time four, the low dose and high dose caffeine groups were not significantly different, And, finally at time five and six the low dose and high dose caffeine groups were significantly different. The high dose caffeine group experienced an increase in horizontal movement while the low dose caffeine group experienced a decrease in horizontal movement (see Figure 4).

Vertical Movement

A main effect for group was found, $F(2, 21) = 4.78$, $p = .020$. The low dose and high dose caffeine groups displayed more vertical movement than the NaCl control group. The caffeine high and low dose groups were not significantly different (see Figure 5).

There was a main effect for time, $F(5, 105) = 10.36$, $p < .001$. There was a decrease in vertical movement from time one to two and from time three to six the amount of vertical movement remained constant (see Figure 5).

Lastly, a significant interaction effect was obtained, $F(10, 105) = 2.17$, $p = .025$. The NaCl control group maintained the lowest amount of vertical movement across time. This was significant at time one and four compared to the low dose and high dose caffeine groups. But, at time one, two, four, five and six only the high dose group experienced significantly more vertical movement compared to the NaCl control group (see Figure 5).

Discussion

The hypothesis was not supported, groups injected with caffeine displayed a decrease in bar presses compared to the control group. This contradicts previous findings which showed that caffeine improved recall memory (Pierard et al., 2015).

Caffeine reduced the response rate (bar press), the most obvious effects for this was in latency; the high dose group had a significantly higher delay before their first response compared to the low dose caffeine group and control (see figure 3). This was consistent with previous literature that found that higher doses of caffeine increased the latency to response (Pettenuzzo et al., 2008). A possible explanation is that bar pressing behaviour reduced due to locomotor activity. Baron, Antonitis, and Beale (1961) found that rats who had more room to explore new environments showed a decrease in bar pressing, as well, previous exposure also reduced bar pressing. They concluded that prior experience with bar pressing for food is affected by prior experience with the stimuli. The self-reinforcing behaviour diminishes overtime and it would decrease the amount of bar presses (Baron et al., 1961).

Yet, this is contrary to the present findings, looking at figure 1 it can be observed that the response rate increased in the post-treatment condition compared to the pre-treatment condition for the control group. That is, bar pressing increased even after all the training trials and baseline testing. However, for the high dose caffeine group the response rate decreased, this indicated that another factor might influence the feeding behaviour in addition to locomotor activity.

Similar to previous studies, caffeine injection increased the locomotor activity of rats (Brockwell & Beninger, 1996; Lau & Falk, 1994). Both the low dose and high dose caffeine groups experienced an increase in locomotor activity compared to the control group. This is in line with findings by Brockwell and Beninger (1996) who indicated that caffeine binds to adenosine receptors. In their study rats were placed in a Skinner box, movement was measured by six pairs of infrared photosensors, the interruptions of the sensors were counted for movement and the time spent in each part of the box was also monitored (Brockwell & Beninger, 1996). The results indicated that unlike A1, A2 antagonist increased locomotor activity. Caffeine increases locomotor activity by acting on adenosine A2 receptors, but at a specific dosage (Brockwell & Beninger, 1996). Similarly, Daly, Shi, Nikodijevic, and Jacobson (1994) found that caffeine causes changes in behaviour due to the antagonism of A1 and A2 adenosine receptors. As such, it was expected that the rats in the present study show an increase in locomotion due to the caffeine interaction with adenosine receptors.

Furthermore, along with the adenosine receptors increasing movement which is potentially why bar pressing was reduced, caffeine is also linked with the dopaminergic pathway and A2 receptors are co-located with dopamine receptors in the striatum (Pattenuzzo et al., 2008). Caffeine and dopamine act on similar reward pathways, the striatum, and the striatum acts on the nucleus accumbens (Kelley, Baldo, Pratt, and Will, 2005). Opioid receptors are located in

the striatum and are widely known for regulating food intake. Enhancing opioid receptor stimulation increases food intake, but, since caffeine is an A2 antagonist it can be thought of as decreasing striatum activity and as a result decreasing opioid receptor activity which could lead to food intake reduction (Pattenuzzo et al., 2008; Kelley et al., 2005). Further research would need to be conducted in order to support this alternate hypothesis on the food intake reduction caused by caffeine.

The latency to response is an indicator that a rats motivation to seek out food decreased. The exact nature of the latency could be due to the increased movement as indicated earlier, but the lack of motivation could also explain why rats had an increase in horizontal and vertical movement. As observed in figure 1, the high dose caffeine group had the highest latency time suggesting that high doses of caffeine suppresses appetite. Further research is required to determine if latency causes an increase in locomotor activity or vice versa.

The present study had several limitations, many studies involved chronic use of caffeine, and the current study only monitored the effects of a one-time caffeine use. This could affect the results as a single exposure may not be enough to produce change, chronic use may increase the influence of caffeine on neurotransmitters and brain regions (Pettenuzzo et al., 2008). Also the type of assessment used for recall memory may not have been adequate, most studies use recognition or word list to test memory, however such assessments are not compatible with animal studies.

Caffeine can be used as a therapeutic for Parkinson's disease and obesity. Findings that suggest caffeine projects on the striatum could help attenuate some of the symptoms experienced in Parkinson's disease (Jankovic, 2008). Parkinson's disease is commonly known for causing tremors at rest. Tremors at rest occur because of hyperactive striatalpallidal neurons (Jankovic,

2008). Kelley et al. (2005) postulated that caffeine, an A2 antagonist, could reduce striatum activity which could potentially reduce tremors at rest. Also, given the appetite reduction effects of caffeine, it can be used in treatment for obesity. Appetite reduction may be due to an increase in serotonin neurotransmitters as suggested by Pettenuzzo et al. (2008). Serotonin is thought to be linked with the state of satiety, it is believed that serotonin controls food intake by acting on the hypothalamus, which regulates body temperature and food intake, to name a few functions (Halford & Blundell, 2000; Meister, 2007).

Future research should aim at determining if caffeine does effect the state of opioid factors as suggested by Pattenuzo et al. (2008). Also, determining the effects of A2 antagonist on the striatum and potential effects on the serotonergic system would provide the empirical support to further research on treatment for Parkinson's disease and obesity. More so, to determine accurately if caffeine has any effect on memory more subjective methods should be used such as recognition and word list recall task on human participants. With the use of verbal communication and more elaborate task, the extent of caffeine's ability to enhance cognition could be determined.

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Figure Captions

Figure 1. Mean rate of response across conditions pre-injection and post-injection. The rat's received a 15 mg/kg (low dose) or 50 mg/kg (high dose) caffeine that were dissolved in 0.9 % saline, the control condition received an injection of the 0.9 % saline only. The rats were placed in an apparatus and observed for six 2 min bins for a total of 12 min. For the pre-treatment trials the groups were not significantly different from each other. But, in the post-treatment trials there was a difference between the groups, the NaCl control post-treatment had higher response rates than the low dose group and high dose group. The low dose group had a higher response rate than the high dose group. Error bars represent the standard error of the mean.

Figure 2. Average response rate across time in min. The rat's received a 15 mg/kg (low dose) or 50 mg/kg (high dose) caffeine that were dissolved in 0.9 % saline, the control condition received an injection of the 0.9 % saline only. The rats were placed in an apparatus and observed for six 2 min bins for a total of 12 min. The NaCl control group had higher response rates than the low dose and high dose groups. The low dose group had a higher response rate than the high dose group. Error bars represent the standard error of the mean.

Figure 3. Latency to first response across conditions. The rat's received a 15 mg/kg (low dose) or 50 mg/kg (high dose) caffeine that were dissolved in 0.9 % saline, the control condition received an injection of the 0.9 % saline only. The rats were placed in an apparatus and observed for six 2 min bins for a total of 12 min. The high dose group had the highest latency time, the NaCl control and low dose groups were not significantly different. Error bars represent the standard error of the mean.

Figure 4. Mean frequency of total horizontal movement across time in min. The rat's received a 15 mg/kg (low dose) or 50 mg/kg (high dose) caffeine that were dissolved in 0.9 % saline, the

control condition received an injection of the 0.9 % saline only. The rats were placed in an apparatus and observed for six 2 min bins for a total of 12 min. The NaCl control group maintained lower horizontal movement than the low dose and high dose caffeine groups. The low dose and high dose caffeine groups were not significantly different. As time elapse, the mean horizontal movement decreased. Error bars represent the standard error of the mean.

Figure 5. Mean frequency of total vertical movement across time in min. The rat's received 15 mg/kg (low dose) or 50 mg/kg (high dose) caffeine that were dissolved in 0.9 % saline, the control condition received an injection of the 0.9 % saline only. The rats were placed in an apparatus and observed for six 2 min bins for a total of 12 min. The NaCl control group displayed less vertical movement than the low dose and high dose caffeine groups. The caffeine high and low dose groups were not significantly different. As time elapse, the mean vertical movement decreased. Error bars represent the standard error of the mean.

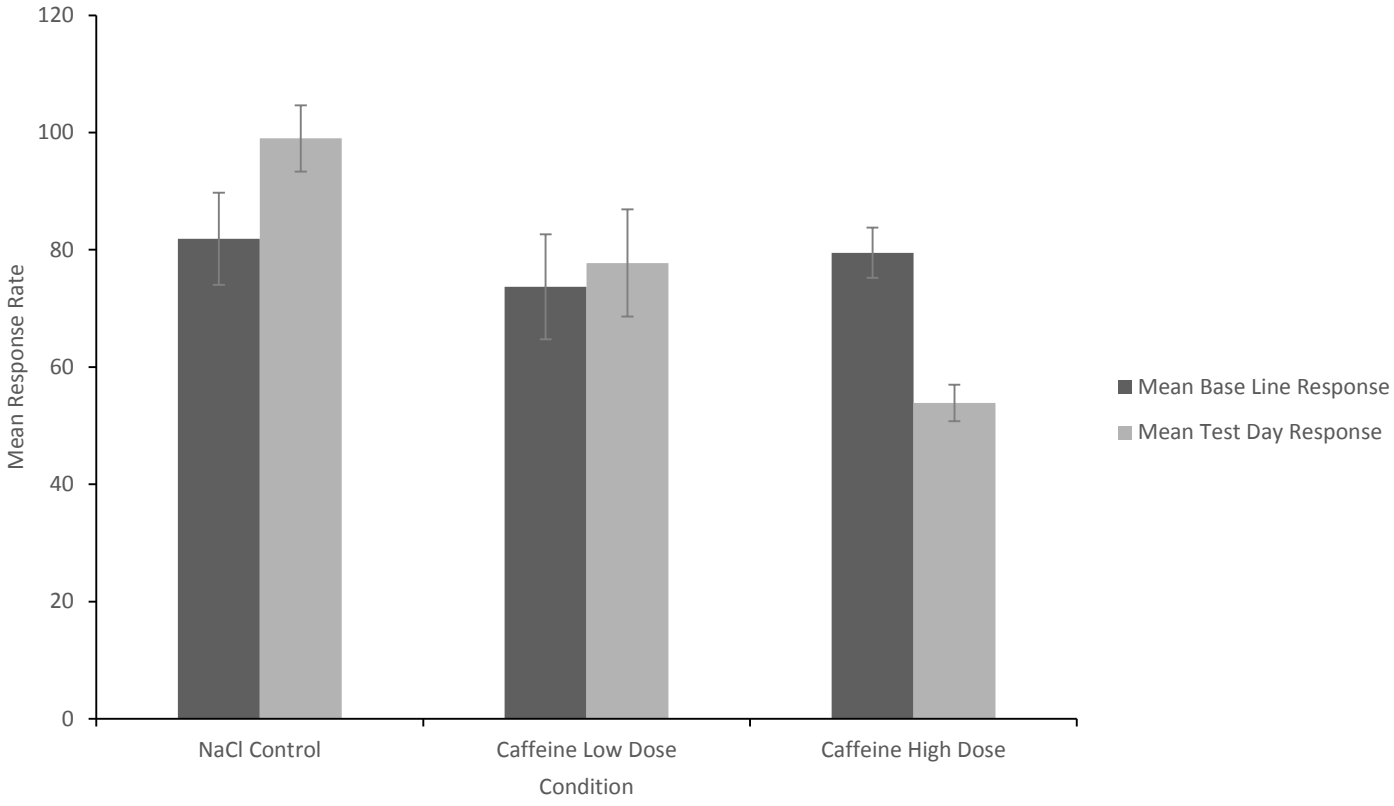

Figure 1. Mean Rate of Response Across Conditions Pre-Injection and Post-Injection

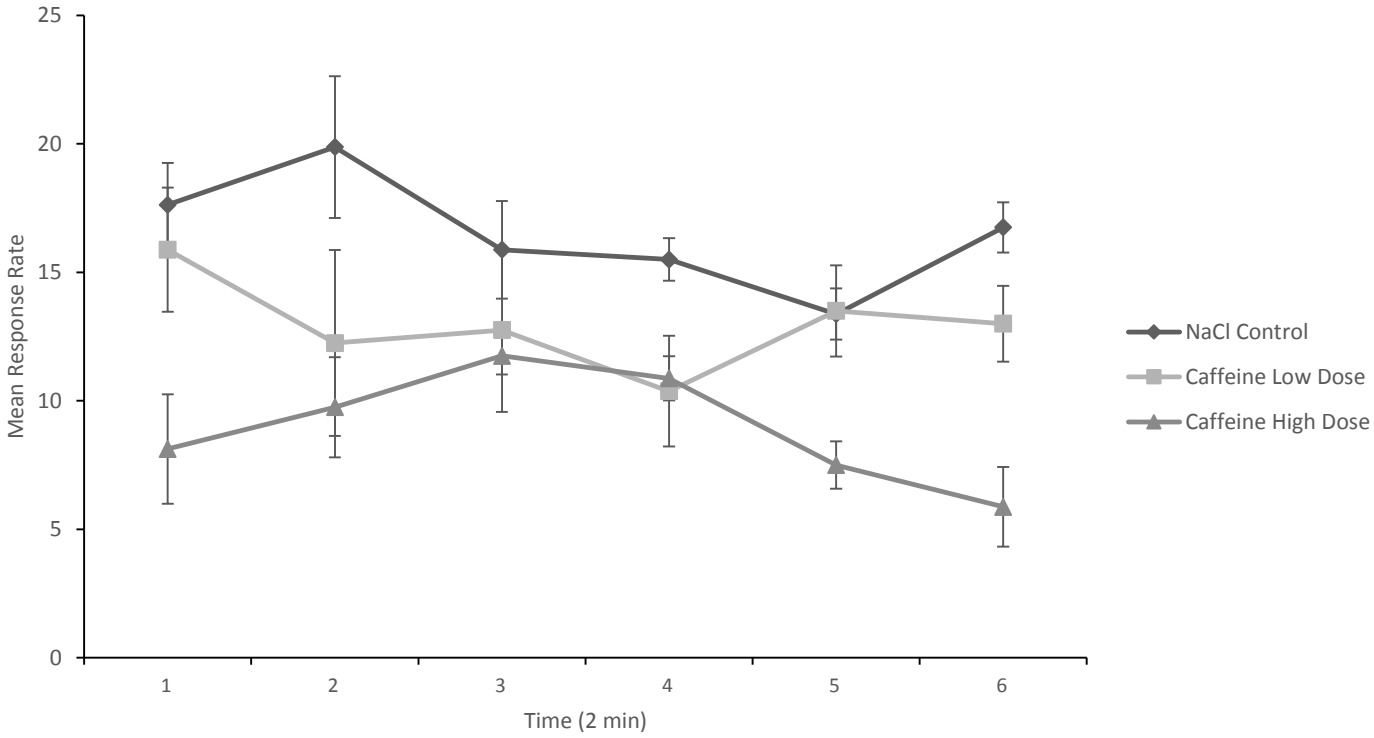

Figure 2. Average Response Rate Across Time (six levels)

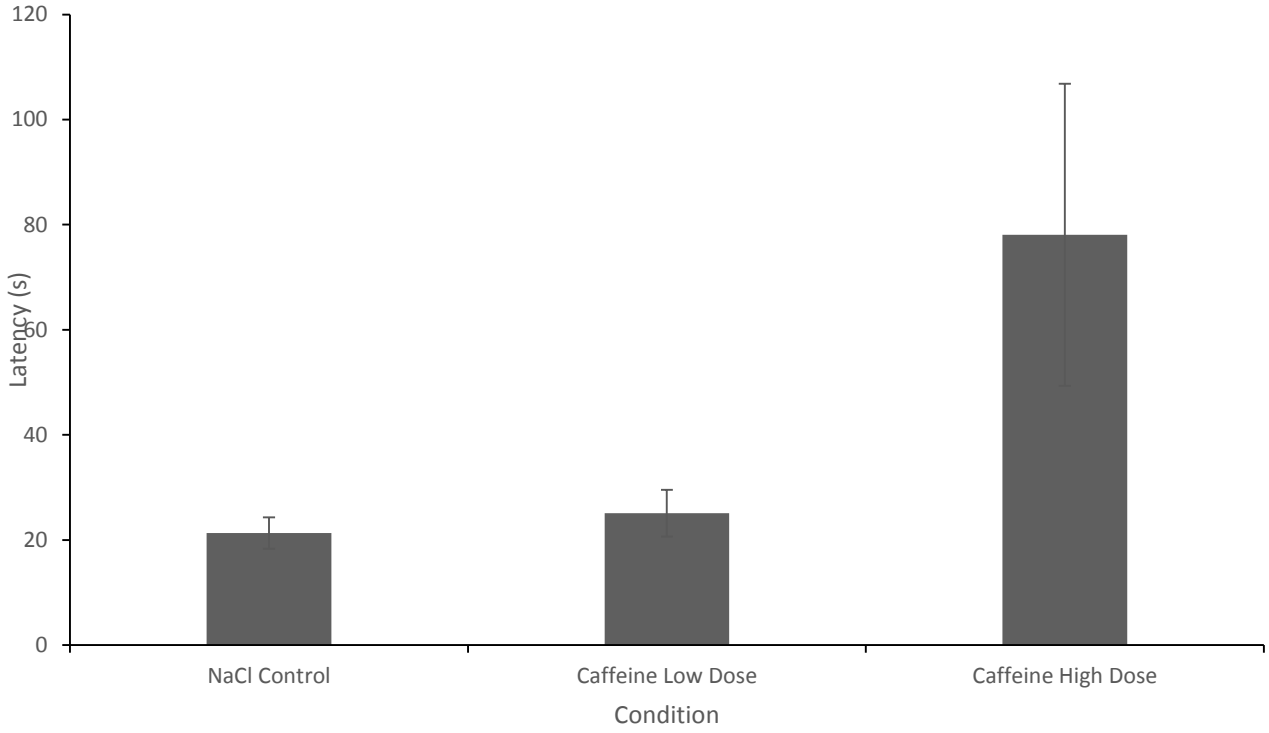

Figure 3. Latency to First Response Across Conditions

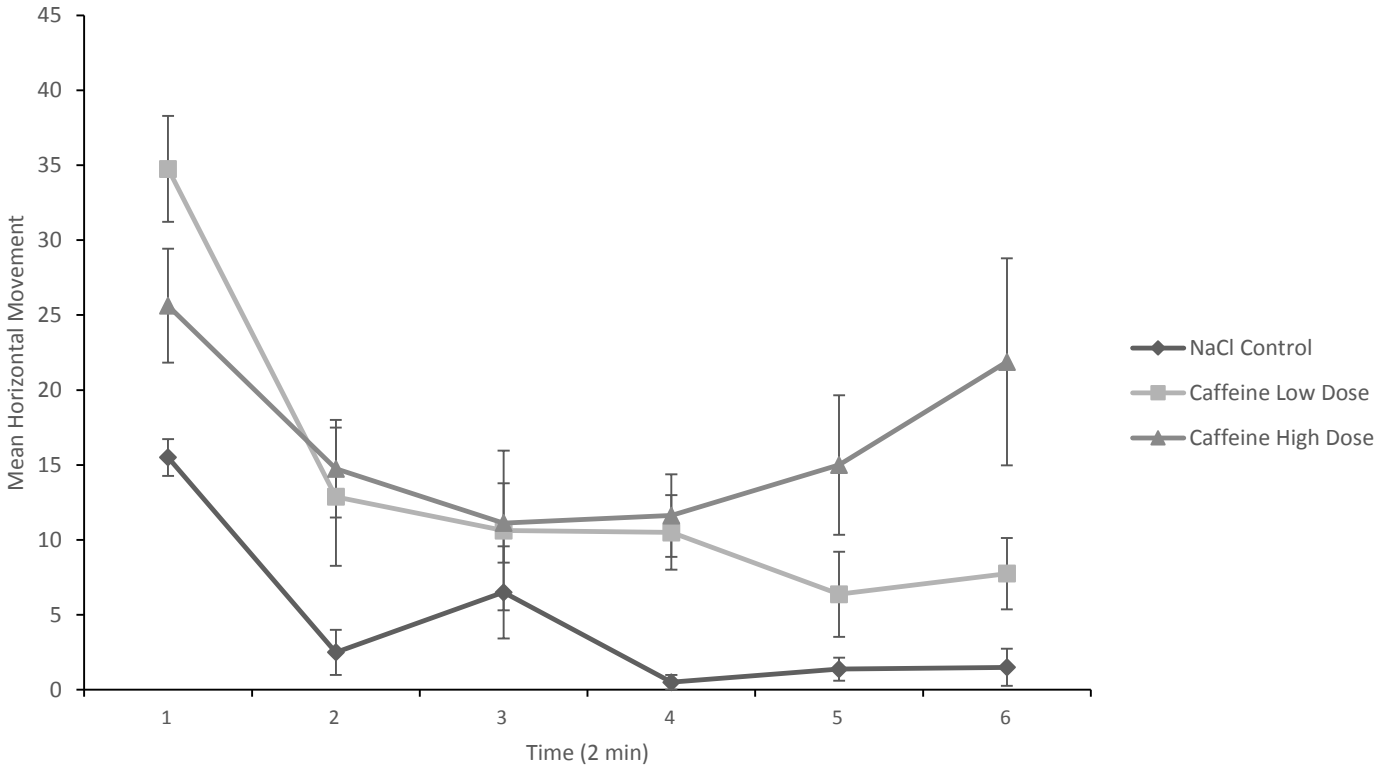

Figure 4. Mean Horizontal Movement Across Time (six levels)

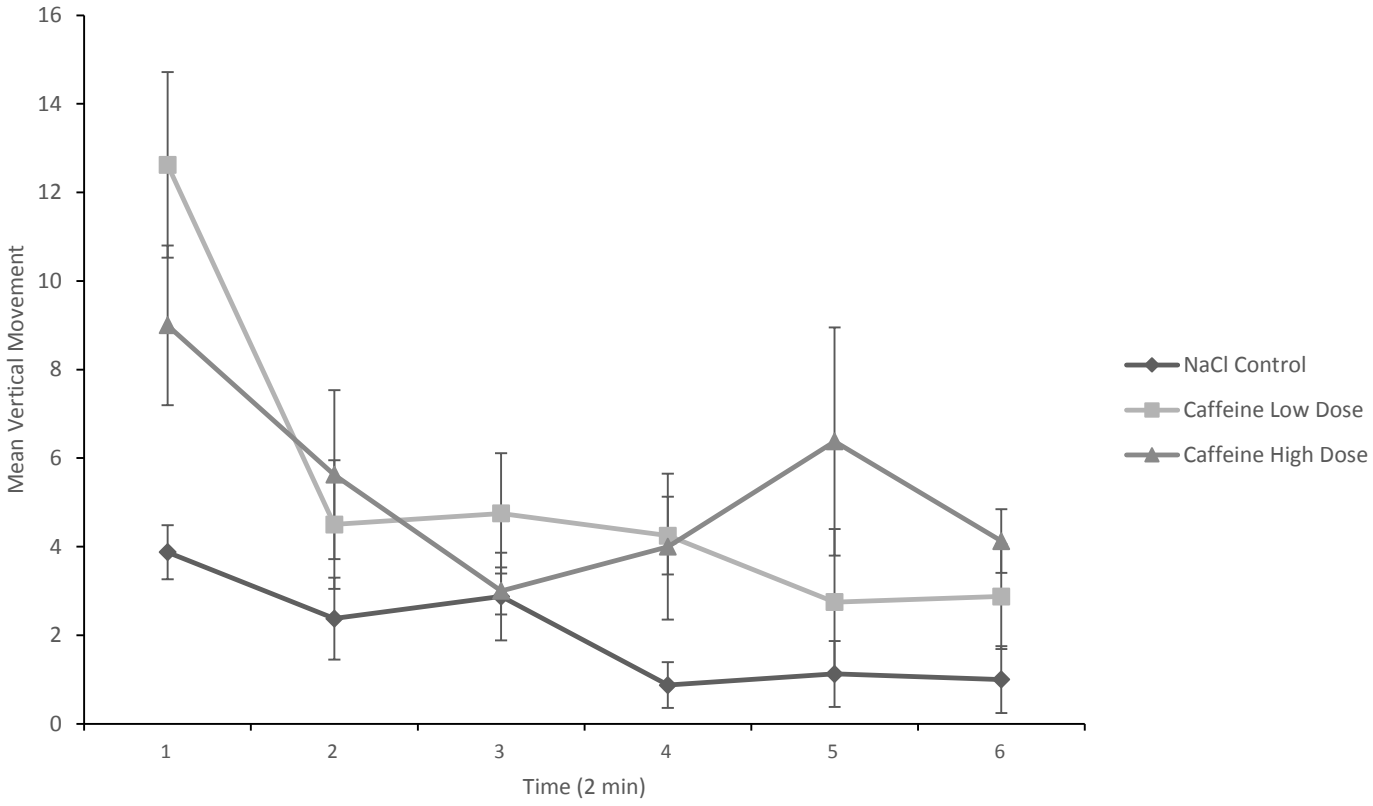

Figure 5. Mean Vertical Movement Across Time (six levels)