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Effects of Lisdexamfetamine on Effort-Related and Consummatory Behaviors Supported by Foods with Varying Degrees of Palatability: Exploration of a Binge-Like Eating Model

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Effects of Lisdexamfetamine on Effort-Related and Consummatory Behaviors Supported by Foods with Varying Degrees of Palatability: Exploration of a Binge-Like Eating Model.

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Abstract: Diets high in sugar or fat are associated with multiple health conditions, including binge eating disorder (BED). BED affects approximately 2% of the US adult population, and occurs more frequently in females. It is important to develop animal models of palatable food consumption, food seeking, and voluntary physical activity that may have relevance for BED and other conditions associated with excessive food intake. The catecholamine uptake blocker and d-amphetamine prodrug lisdexamfetamine (LDX) is used to treat BED. The present experiments studied the effect of LDX on food intake and two effort-based choice tasks in female Wistar rats. In experiment one, three groups of rats received different food exposure conditions in the home cage randomly spread over several weeks: the chocolate exposure group (CE; brief access of chocolate and additional lab chow, n=15), a lab chow exposure (LChE) group was given additional access to lab chow (n=8), and a third group was given empty food dishes (n=7). In tests of food intake under non-restricted conditions, LDX (0.1875-1.5 mg/kg IP) significantly reduced intake of both chocolate and chow in the CE group. In the LChE group, there was a trend towards reduced chow intake induced by LDX. All rats were trained on a Progressive Ratio/chow feeding choice task, in which they had a choice between working for high carbohydrate chocolate flavored pellets by lever pressing vs. approaching and consuming a concurrently available lab chow. The LChE group and the empty food dish group were combined to create one control group (n=15). There was a significant overall dose-related suppressive effect of LDX on lever pressing but no group difference, and no dose x group interaction. LDX significantly decreased chow intake in the CE group, but not in the control group. In Experiment two, rats (n=8) were given brief access of chocolate in the home cage randomly spread over

several weeks. They were then trained on a novel T-Maze task in which they had a choice to eat freely available chocolate or run on a running wheel. LDX (0.09375-1.5 mg/kg IP) significantly reduced intake of chocolate, and a trend towards reduced running wheel activity was seen. In conclusion, LDX affected both food intake, food-reinforced operant behavior, and running wheel activity.

Introduction

Disordered eating, such as what is seen in anorexia nervosa, bulimia nervosa, and Binge Eating Disorder (BED), is associated with both physical and psychological disorders. Individuals with eating disorders have increased risks for cardiovascular problems, neurological dysfunction, decreased immune function and increases in anxiety (Johnson et al. 2002). This can lead to increased levels of diabetes and psychiatric disorders later in life (Johnson et al. 2001). Mortality due to eating disorders is very high in part to factors such as increased rates of smoking, alcoholism and suicide within the population (Herzog et al. 2000). Implications on reproductive health in individuals with a history of EDs have also started to come to light, including infertility and increased risk of miscarriage (O'Brien et al. 2017).

With the release of the DSM-V in 2013, BED was recognized as one of three specified feeding and eating disorders. Before this time, people with BED were considered as having an "eating disorder not otherwise specified." BED's representation as a distinct category distinguishes it from overeating, which is a major public health concern today, but does not encompass all of the psychological symptoms associated with BED such as the feeling of a lack of control in food consumption. This leads an individual to consume large amounts of food more rapidly than normal in a short period of time, typically consuming food until uncomfortably full, even if the person is not hungry. These episodes occur at least once a week during a 3 month period, and individuals report being extremely distraught over them (DSM-V; APA 2013). A survey completed by the World Health Organization (WHO) conducted across 14 different countries found BED to have a lifetime prevalence rate of 1.4%, with a typical onset

being in the late teens to early 20's (Kessler et. al 2013). In the United Sates alone, BED has a lifetime and 12-month prevalence rate of 0.85% and 0.44% respectively. (Udo & Grilo 2018). Occurrence is higher in women (3.5%) than in men (2.0%) and has been shown to be relatively independent of weight but is more common among overweight and obese individuals (Bruce and Wilfley 1996, Hudson et al. 2007). Diets high in fat and sugar have been linked to increases in obesity, type 2 diabetes, hypertension and cardiovascular disease (Guh at el. 2009).

Treatment outcomes for BED are variable based on the treatment used (Wilson 2011). Common treatments for BED include both psychological and pharmacological interventions. Cognitive behavioral therapy and guided self-help have shown the most promise in terms of psychological treatments (Ghaderi et al. 2018). Pharmacologically, many classes of drugs have been looked at, including antidepressants such as the SSRI fluoxetine (Prozac), anti-obesity, antiepileptic, and anti-addiction medications (Ghaderi et al. 2018). The *d*-amphetamine pro-drug lisdexamfetamine (LDX; Vivanse), a common medication for treating attention deficit hyperactivity disorder, was approved for the treatment of moderate to severe BED in 2015. As a pro-drug, LDX has a slower on set and longer lasting effects (Heal et al. 2013). LDX, due to l-lysine being covalently bound to d-amphetamine, is inactive until it is metabolized within the body, more specifically within the red blood cells (Pennick 2010, Heal et. al 2013).

Animal models of binge-like feeding behavior have been developed (Hagan et al. 2002, Colantuoni 2003, Avena & Hoebel 2003, Rada et al. 2005, Boggiano & Chandler 2006, Corwin & Wojnicki 2006, Avena et al. 2004, 2006, Heyne et al. 2009; Vickers et al. 2015; Smith et al. 2015; Smail-Crevier et al. 2018), which involve induction of

excessive eating of a highly palatable food. After inducing chocolate binging behavior in rats, Vickers et al. (2015) reported that LDX can reduce chocolate intake in a dose-dependent manner during binging sessions. Vickers et al. (2015) highlighted the effectiveness of LDX at attenuating consumption, but did not look at effort-related aspects of food seeking or reinforced behavior. The following experiments were intended to address this.

Experiment One: Operant Conditioning

One characteristic of motivated behavior is that a high degree of effort is often expended in order to achieve a goal. Behavioral tasks have been developed to look at effort-related aspects of motivated behavior in both humans and animals (Salamone et al. 1991, 1994, 2016; Treadway et al. 2009; Randall et al. 2012; Sommer et al. 2014; Hosking et al. 2015). These tasks include maze procedures that require high physical activity to obtain reinforcers, operant procedures such as progressive ratio (PROG) schedules that require increasing levels of work output to obtain reinforcement, and effort-based decision making tasks (Salamone et al. 2016). Nasser et al. (2008) studied humans with and without a BED diagnosis using a food-reinforced PROG operant task. Before the task, subjects were either given water or a drinkable meal. Nasser et al. (2008) reported that individuals without BED had a significant reduction in the amount of work they were willing to put into achieving their food reward after receiving the meal drink, whereas individuals diagnosed with BED did not. These results indicated that people with BED are not motivated by hunger.

Because it is critical to employ animal models to study phenomena related to binge eating on palatable foods, the present experiments studied the effect of LDX on food intake and food-reinforced effort-based choice in female Wistar rats. Three groups of rats received different food exposure conditions in the home cage randomly spread over several weeks: the chocolate exposure group (CE; brief access of chocolate and additional lab chow, n=15), a lab chow exposure (LChE) group was given additional access to lab chow (n=8), and a third group was given empty food dishes (n=7). Chocolate intake in female Wistar rats was studied based upon the Vickers et al. (2015) procedure. After having experience with their respective exposure conditions, the rats were tested for the effects of LDX on intake of the different foods (chocolate and standard lab chow) as well as effort-based choice using the PROG/chow feeding choice procedure that has been developed in our laboratory (Randall et al. 2012, 2014, 2015; Yohn et al. 2016 a,b,c). With this task, rats had a choice between working for preferred high carbohydrate chocolate flavored pellets by lever pressing on the PROG schedule vs. approaching and consuming a concurrently available but less preferred lab chow. These procedures allowed for investigation of the effects of LDX on food intake as well as the tendency to work for food.

Methods

Animals

Singly-housed, adult female, Wistar rats (N = 30 Charles River) weighing between 226-250 grams on arrival were food restricted to 85% of their free feeding weights for 16 weeks allowing for modest growth. Water was available ad libitum.

Starting at 17 weeks, rats were given food ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee.

Operant Training

Using Med Associates operant chambers (28 × 23 × 23 cm), rats were trained to lever press for high carbohydrate pellets on a FR1 schedule for 3 weeks. Training then began on the PROG task for 8 weeks (30 min sessions, 5 days a week) after which freely available lab chow was placed in the box during their session (PROG/chow choice task) to give them an option of either lever pressing for pellets or approaching and consuming the lab chow. They remained on the PROG/chow feeding choice task for the remainder of the experiment. Rats were weighed prior to each operant task. At week 11 of training on the PROG/chow choice task, the rats were transitioned from plain high carbohydrate pellets to chocolate flavored high carbohydrate pellets for the remainder of the experiment. This was done because of previous studies that used chocolate flavored pellets for operant conditioning studies in rats tested in a binge eating model (Smith et al. 2015).

Development of chocolate binge-consumption behavior

The rats were randomly assigned to 3 groups: chocolate exposure group (CE; n=15), lab chow exposure group (LChE; n=8), and empty food dish group (n=7). In the home cage, a one-day, one-hour exposure to two ceramic dishes took place before the introduction of chocolate. The exposure schedule mirrored that used by Vickers et al.

(2015), with rats receiving exposure of 1 of 3 different conditions based on their assigned group on days 1, 2, 4, 6, 7, 9, 12, 14, 15, 18, 23 and 28. On exposure days, 2 ceramic dishes were placed in the home cage of each rat for 2 hrs. For the first exposures, the chocolate exposure group was given access to one dish containing chocolate, and a second, empty dish. Starting on day 7, the chocolate exposure group had one dish containing chocolate and another containing lab chow. The lab chow exposure group had a dish containing lab chow and one empty dish, and finally, the empty food dish group had two empty dishes. After exposure day 28, exposure continued on a randomized schedule with 2 sessions happening each week. Starting on day 58, the 2 weekly sessions were split between a morning session and an afternoon session. Weights of both chocolate and lab chow were taken before and after each session.

Administration of Lisdexamfetamine (LDX) during food exposure sessions

Doses of LDX were based on experiments completed previously (Yohn et al. 2016b). Starting on week 15 of exposure, IP injections of 0.1875, 0.375, 0.75, 1.5mg/kg or vehicle (saline) were given to rats once per week in a randomly varied order 60 minutes prior to testing. Drug administration only occurred during afternoon exposure sessions. Weights of both chocolate and lab chow were taken before and after each session to determine the effect of LDX on consumption.

Administration of Lisdexamfetamine during operant behavior sessions

Starting on week 20 of exposure, LDX administration of 0.1875, 0.375, 0.75, 1.5mg/kg or vehicle (saline) was given to rats once per week in a randomly varied order 60 minutes prior to their operant behavior session. Amount of lever pressing and lab chow consumption were both recorded to determine the effect LDX had on both lever pressing and chow intake.

Statistical Analyses

A repeated measures of analysis of variance (ANOVA) was used to evaluate the effect of LDX on the amount of chocolate and chow in grams consumed during a food intake session. Planned comparisons were used to compare each treatment to the VEH group. Effect sizes of consumption amounts were determined using partial eta square values. A factorial ANOVA was used to compare the CE group versus the control group during the 30-min operant session for both lever pressing (LP) and chow consumption.

Results

Acquisition of Chocolate Binging Behavior, and Body Weight Data

Analysis of the initial acquisition period for chocolate binging behavior was performed using a repeated measures ANOVA. There was an overall significant effect of binge session [$F(11,154) = 27.379$, $p < 0.001$) Fig. 1]. Chocolate intake increased dramatically over the first several sessions, leveled off, then decreased slightly when chow was introduced. Significant linear [$F(1,14) = 89.333$, $p < 0.001$)] and quadratic [$F(1,14) = 104.153$, $p < 0.001$)] contrasts were seen, leading to separate analyses of the sessions before and after chow was introduced. A significant increase across binge

sessions was seen before chow was introduced (days 1-7 [$F(4,56) = 32.421$, $p < 0.001$]) and a small but significant decrease was seen after chow was added to the binge sessions (days 9-28 [$F(6,84) = 3.892$, $p = 0.002$)]). Repeated measures ANOVA analysis of body weights (Figure 2) over the course of the experiment found a significant overall effect due to week [$F(23, 644) = 195.156$, $p < 0.001$)] but no significant difference between the CE and control groups [$F(1, 28) = 0.119$, $p = 0.733$)], and no interaction ($F(1,23) = 0.738$, n.s.). This indicated that despite the occasional exposure to chocolate, body weight was not affected in the CE group.

Effect of Lisdexamfetamine administration on intake during exposure sessions

Repeated measures ANOVA showed a significant overall effect of LDX on intake of chocolate [$F(4,56) = 7.713$, $p < 0.001$) Fig 3.] and lab chow [$F(4,56) = 11.475$, $p < 0.001$ Fig. 3] in the CE group. Planned comparisons analysis found LDX significantly reduced chocolate consumption at the highest dose of LDX ($p < 0.05$) and significantly reduced chow consumption at the two highest doses ($p < 0.05$) when compared to the VEH treatment. For the LChE group, the ANOVA approached significance [$F(4,28) = 2.533$, $0.05 < p < 0.1$) Fig 4.], with a strong tendency towards a decrease in chow intake at the highest dose. Table 1 lists the partial eta squared effect sizes for the actions of LDX on intake of chocolate and chow across both groups. Moderate to large effect sizes for all three ANOVAs illustrate a strong trend towards a reduction of consumption in both groups with very large effect sizes (partial eta squared > 0.40) at the highest dose of LDX in all three conditions studied.

Effect of Lisdexamfetamine administration during operant sessions

Factorial ANOVA revealed a significant overall effect of LDX treatment on lever pressing (PROG/chow choice task) [$F(4,112) = 10.739$, $p < 0.001$) Fig. 5], but no significant group difference, and no significant treatment x group interaction. For chow consumption, there was a significant overall treatment effect [$F(4,112) = 5.905$, $p < 0.001$ Fig. 6]. A group difference was trending towards significance [$F(1, 28) = 2.172$, $p = 0.072$] but a significant treatment x group interaction [$F(1, 28) = 4.561$, $p < 0.05$] was seen. Planned comparisons revealed that LDX significantly reduced lever pressing at the three highest doses of LDX ($p < 0.001$) in both groups and significantly reduced chow consumption at the three highest doses ($p < 0.05$, 0.001) of the CE group when compared to the VEH treatment. There was no reduction of chow intake in the combined control group.

Experiment Two: T-Maze Task

The development of novel animal models in the study of motivation are necessary in order to continue gaining new insight into this complex aspect of behavior. Tasks similar to what was highlighted in experiment one use food as an overall motivating factor. Complications using these types of methods can develop when a pharmacological agent known to produce appetite suppressing effects is used during the task. A question arises about whether or not the observed behavioral outcome is due to factors that are specific to the particular motivational stimulus. Running on a running wheel (RW) has been shown to be an intrinsically reinforcing activity in rats (Kagan and Berkun 1954, Collier and Hirsch 1977, Pierce et al. 1986, Iversen 1993),

subject to multiple types of reinforcement schedules. A recent study completed in mice found that when given the option to run on a RW or consume freely available lab chow, mice consistently chose the RW (Correa et al. 2016). With all of this in mind, in order to study the effects of LDX using a task that involved a food reinforcer as well as a non-food reinforcer, a T-Maze task for rats was developed to pit the desire to consume food against the desire for voluntary physical activity in terms of RW activity.

Using a model similar to what has been developed in mice (Correa et al. 2016), a RW was placed in one arm of a standard T-Maze, with a palatable food (Cadbury's Milk Chocolate) placed in a ceramic bowl in the other. Rats were then placed in the middle of the maze and given 30 minutes to choose between going back and forth between the RW and palatable food. LDX was administered once chocolate consumption and RW activity were stable in terms of baseline levels. This new procedure allowing the animal to choose between two different motivating factors can be used to tease apart the changes in behavior that can become muddied with a single motivating factor.

Methods

Animals

Singly-housed, adult female, Wistar rats (N = 8 Charles River) weighing between 226-250 grams on arrival were given water and food ad libitum.

Development of chocolate binge-consumption behavior:

Similar to the procedure used during the operant experiment, a one-day, one-hour exposure to a ceramic dish took place in the rat's home cage before the

introduction of chocolate. Rats received exposure of chocolate on days 1, 2, 4, 6, 7, 9, 12, 14, and 15. On exposure days, 1 ceramic dish was placed in the center front of the home cage of each rat for 1 hour. Weight of chocolate was taken before and after each session. Amounts given were determined by consumption of the rat in their prior session. Once binge-like eating behavior was established, chocolate was no longer given in the home cage but was only offered during the T-Maze task.

T-Maze Apparatus

A Plexiglas T-maze apparatus consisting of a start arm (29X21X21 cm) and two test arms (99X32X59 cm) was utilized for this task. Within the maze, a clear box (47.6X25.9X20.9 cm) with a hole (10.3 cm diameter) cut in the front was placed in either the right or left arm. This box housed the RW (Starr Life Sciences Corp.) apparatus with an attached counter to record RW rotations during sessions. A wall (32X59 cm) with an entry hole was placed in front of the box to keep the rats from moving around the box during early stages of training. The opposite arm contained a ceramic bowl (9 cm diameter) (PetCo) with ground milk chocolate (Cadbury's Dairy Milk).

T-Maze Training

RW Training:

Rats were first introduced to the RW by being placed directly into the box with the entry hole blocked so they only had access to the RW area for two sessions. On the third session, the side containing the bowl was blocked off and rats were placed in the middle of the maze facing the wall. This required the rat to use the entry hole of the RW box. On the final session the maze was open for the rat to explore both arms with an empty ceramic bowl placed opposite to the RW. Each session lasted 30 minutes.

Chocolate Training:

After the induction of chocolate binge behavior, rats were blocked in the arm containing the ceramic bowl and chocolate for two sessions. Sessions 3, 4, and 5, of T-Maze chocolate exposure, the rat was placed in the maze with the RW entry hole blocked off, which allowed access to the open maze but entry only to the chocolate arm. Each session lasted 30 minutes.

Administration of Lisdexamfetamine (LDX) during T-Maze sessions:

Doses of LDX were based on experiments completed previously (experiment one). Once consistent RW activity and chocolate consumption was reached in the T-Maze, administration of 0.09375, 0.1875, 0.375, 0.75, 1.5mg/kg or vehicle (saline) was given to rats in a randomly varied order once per week, 60 minutes prior to T-Maze entry. Weights of chocolate were taken before and after each session, and RW rotations (complete rotations) were recorded, to determine the effect LDX had on both chocolate intake and RW activity. Video recordings of sessions were used to determine time spent

actively or passively interacting with the RW, time spent eating, time spent doing neither of those activities, and number of side changes and rears.

Statistical Analyses

A repeated measures analysis of variance (ANOVA) was used to evaluate the acquisition of chocolate binging behavior and the effects of LDX on the percent baseline amount of chocolate in grams consumed during a food intake session and percent of the averaged weekly baseline RW activity. Planned comparisons were used to compare each treatment to the VEH group.

Results

Acquisition of Chocolate Binging Behavior

Analysis of the acquisition period of chocolate binge-like eating behavior (Figure 7) using a repeated measures ANOVA found an overall significant effect of binge session [$F(8, 56) = 8.214$, $p < 0.001$)]. A significant linear [$F(1, 7) = 16.173$, $p = 0.005$)] contrast was seen highlighting the increase in chocolate consumption across sessions.

Effect of LDX administration during T-Maze sessions

A repeated measures ANOVA of the LDX test sessions in the maze (Figures 8 and 9) revealed a significant overall effect of LDX treatment on percent baseline chocolate consumption [$F(5, 35) = 7.879$, $p < 0.001$)], but no significant effect on percent baseline RW activity [$F(5, 35) = 1.254$, $p > 0.3$]. Planned comparisons revealed

that LDX significantly reduced chocolate consumption at all but the lowest dose of LDX ($p < 0.05$) when compared to the VEH treatment.

Discussion

The overall purpose of these studies was to characterize the effects of the *d*-amphetamine pro-drug LDX on intake of chocolate and lab chow, as well as two different effort-based choice tasks, one entirely based upon food reinforcement and the other comparing two different options (i.e. T-maze choice of RW vs. chocolate intake). These studies were done in female rats that were previously exposed to different types of food (e.g. chocolate or chow) under conditions that mimic previously used binge-like eating models (Vickers et al. 2015). Clinical research shows the prevalence of BED is greater in the female population (Dingemans et. al 2002, Kessler et. al 2013, Hutson et. al 2018) with twelve-month and life time prevalence rates being 0.8% and 2% in males and 1.6% and 3.5% in females respectively (Hudson et al. 2007). Therefore, it was deemed important to conduct this research in female rats in order to better mimic what is seen in the human population.

Our results for chocolate binge consumption and body weights from experiment one are consistent with what was reported in the literature in studies employing a similar rat model (Corwin and Wojnicki 2006, Vickers et al. 2015). Random, intermittent, short periods of access of chocolate led to a robust and significant increase in chocolate intake over the repeated sessions (Fig. 1). This was attenuated slightly when chow was added to the binge sessions and with the removal of food restriction (appendix Fig. 1). This attenuation of chow intake was also seen in the LChE group upon the removal of

food restriction but in a much larger degree than that in the CE group (appendix Fig. 2). The significant increase in body weights for both CE and control animals over the course of the study illustrates the natural growth of the rats over time, but there was no significant difference in body weights between the CE and control rats, and no significant group x week interaction (Figure 2), which is consistent with much of the literature on binge-like eating models in rodents (Hagan et al. 2002; Corwin et al. 2011, 2016; Vickers et al. 2015; Smail-Crevier et al. 2018). In this regard, it is worth emphasizing that although BED is more commonly diagnosed in obese individuals, it also is seen in individuals within the normal BMI range (Bruce and Wilfley 1996; Hudson et al. 2007).

The d-amphetamine pro-drug LDX became an approved treatment for BED in 2015. In the case of LDX, d-amphetamine (the active moiety) is covalently bound to the amino-acid L-lysine (inactive) via an amide linking group, and the d-amphetamine only becomes active once it is metabolized (Heal et. al 2013). This leads to a slow release of d-amphetamine and longer lasting effects when compared to d-amphetamine on its own. In the clinical setting, LDX has been shown to significantly reduce the number of binge eating days/week (McElroy et al. 2016). The results of the present food intake studies showed that LDX produced a robust suppression of both chocolate and chow intake in the CE group (Fig. 3). There was a trend towards a reduction in lab chow intake during the binge sessions in the LChE group (Figure 4), but due to a small sample size (n=7) this effect did not reach statistical significance. Nevertheless, it can also be seen in Table 1 that the effect size for the action of LDX in the LChE group was quite large, and roughly comparable to that seen in the CE group.

In terms of results from the operant experiment, there was an overall significant reduction of lever pressing induced by LDX that was seen across both groups. There was an overall significant effect of LDX on chow intake, however, there also was a significant group x drug treatment interaction. It appears that a major source of the interaction was due to the high consumption of chow in the CE group compared to the control group after VEH treatment. This difference allowed for a significant reduction in lab chow consumption in the CE group, but there may not have been a significant reduction in the control group due to a floor effect. Given this pattern of results, it is reasonable to suggest that with the induction of the binging behavior in the CE group, these animals also consumed more chow during the operant sessions compared to the control group, and that this led them to be more sensitive to the effects of LDX in that setting.

It is important to point out that these drug studies were performed under conditions in which the animals were not food restricted. Food restriction is generally useful in order to have rats emit large numbers of responses for food reinforcement. Thus, food restriction was used during the initial portion of the operant training. However, it was decided to remove the rats from food restriction after initial acquisition in order to better mimic what is seen clinically in people with BED, as well as in other rat models of binging behavior. Once food restriction was removed, we observed lower levels of both lever pressing and food intake in the rats (appendix Figs. 3 & 4). It is possible that more robust results in the operant study would have been seen if the rats had continued on food restriction.

The induction of the binge-like consumption of chocolate in the T-maze study varied slightly from what was seen in the operant task. This was due to the fact that once the T-Maze task was underway, rats only received chocolate in the maze. The rats completing the RW task were also not food restricted at any point of the study. Even with these variations from the protocol in experiment one, a robust increase in chocolate intake was seen (Fig. 7) with the use of random, intermittent, short periods of access to chocolate. This finding adds to the already established literature that food restriction is not an important factor in the development of binging behavior in animal models (Avena et al. 2006; Corwin & Wojnicki 2006), and also what is seen in the BED human population, with whom hunger is not always a driving factor of binging episodes (Brownley, Berkman, Sedway, Lohr, & Bulik, 2007; Davis et al., 2007).

The purpose of developing a novel task that pits two reinforcing behaviors against one another was intended to clarify some of the factors mediating the actions of LDX. Due to the appetite suppressant effects previously mentioned, it becomes difficult to say the results we observed in the operant task, which is an entirely food motivated task, are not simply due to the fact the rat is no longer hungry. The results from the RW task showed a similar pattern of chocolate intake seen in both the strict binge consumption and operant tasks of experiment one, with a significant reduction of intake in a dose dependent manner (Fig. 8). Percent of baseline analyses were used due to the fact that the rats varied greatly from one another in their intakes. It was not surprising that there was a reduction in intake of chocolate given the results of experiment one and the previously mentioned literature on LDX's ability to attenuate

binging behaviors in both rat models and human participants (Hagan et al. 2002; Corwin et al. 2011, 2016; Vickers et al. 2015; Smail-Crevier et al. 2018; McElroy et al. 2016).

Amphetamine exposure induces differential alterations across multiple activities based on the baseline level of behavior and the dose given. Rate-dependency is a drug-induced change in operant behavior in which schedules of reinforcement with high rates of responding will yield decreases in responding with the administration of a stimulant, while those with low baseline rates tend to increase (Dews 1958). RW activity was high in this group of rats, with an average of 252± 6.33 rotations of the RW in 30 minutes. Lyon and Robbins (1978) highlighted the fact that different doses of psychomotor stimulants elicit different behavior with low doses producing an increase switching from one thing to another with high doses resulting in perseveration and stereotypy. LDX administration had no significant effect on RW activity but there was a trend towards a reduction of activity as doses of LDX increased (Fig. 9). Due to the high activity prior to drug exposure, it appears this task may be subject to rate-dependent effects, and it is also possible that, with the increasing doses of LDX, rats began engaging in other behaviors (i.e. increased exploration of maze, rearing, etc.) that lead to a decrease in the amount of RW activity. Serwatkiewicz at al. (2000) found a similar phenomenon with rats exposed to d-amphetamine. They found a significant decrease in RW activity at the highest doses of d-amphetamine administered, and concluded that this was due to an increase of stereotypic behavior. It is likely that with a higher N, the reduction in RW activity would have been found to be significant.

Overall, it is clear that LDX reduces intake of the highly palatable chocolate. However, it also seems evident that the effect of LDX is not limited to chocolate

consumption. Thus, a part of the effect of LDX appears to be a general suppression of food intake, as indicated by the reductions in chow intake that also were seen in experiment one. Moreover, LDX suppressed food-reinforced PROG responding across both the chocolate exposure and control groups. Comparing this with the results seen in the T-Maze task, this reduction in lever pressing could also be attributed to an increase in alternative activities, similar to the decrease in RW activity seen in experiment two. Previous studies have shown that LDX suppresses chow intake in rats in the context of studies using binge-like eating procedures (Vickers et al., 2015), although this point was not highly emphasized in that paper. The active moiety in LDX, d-amphetamine, has been shown to induce appetite suppressant effects, thus leading to its use as a weight loss drug for several decades (Coleman 2005). This is consistent with the fact that a general suppression of appetite has been reported as a side effect of using LDX to treat BED in humans (Citrome 2015, McElroy et al. 2016; Ward and Citrome 2018).

Figures

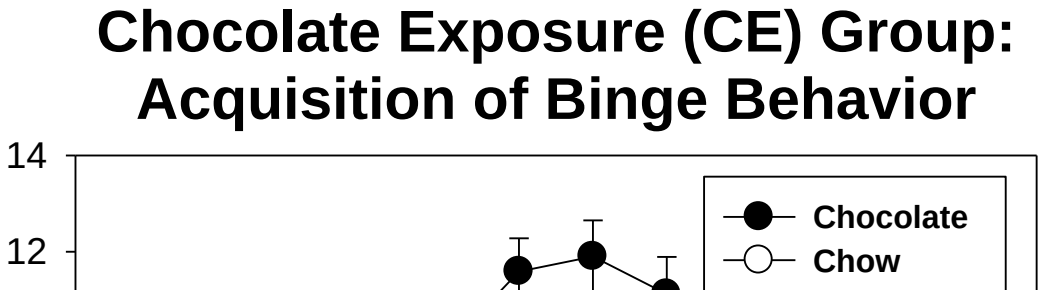


Fig. 1. Chocolate and Chow intakes for CE group over the course of acquisition of binge eating behavior (Mean ± SEM intake in grams). Coinciding with introduction to lab chow during operant task, chow was introduced during 6th binge session. An increase in chocolate intake was seen before the addition of chow at which put intake was slightly reduced.

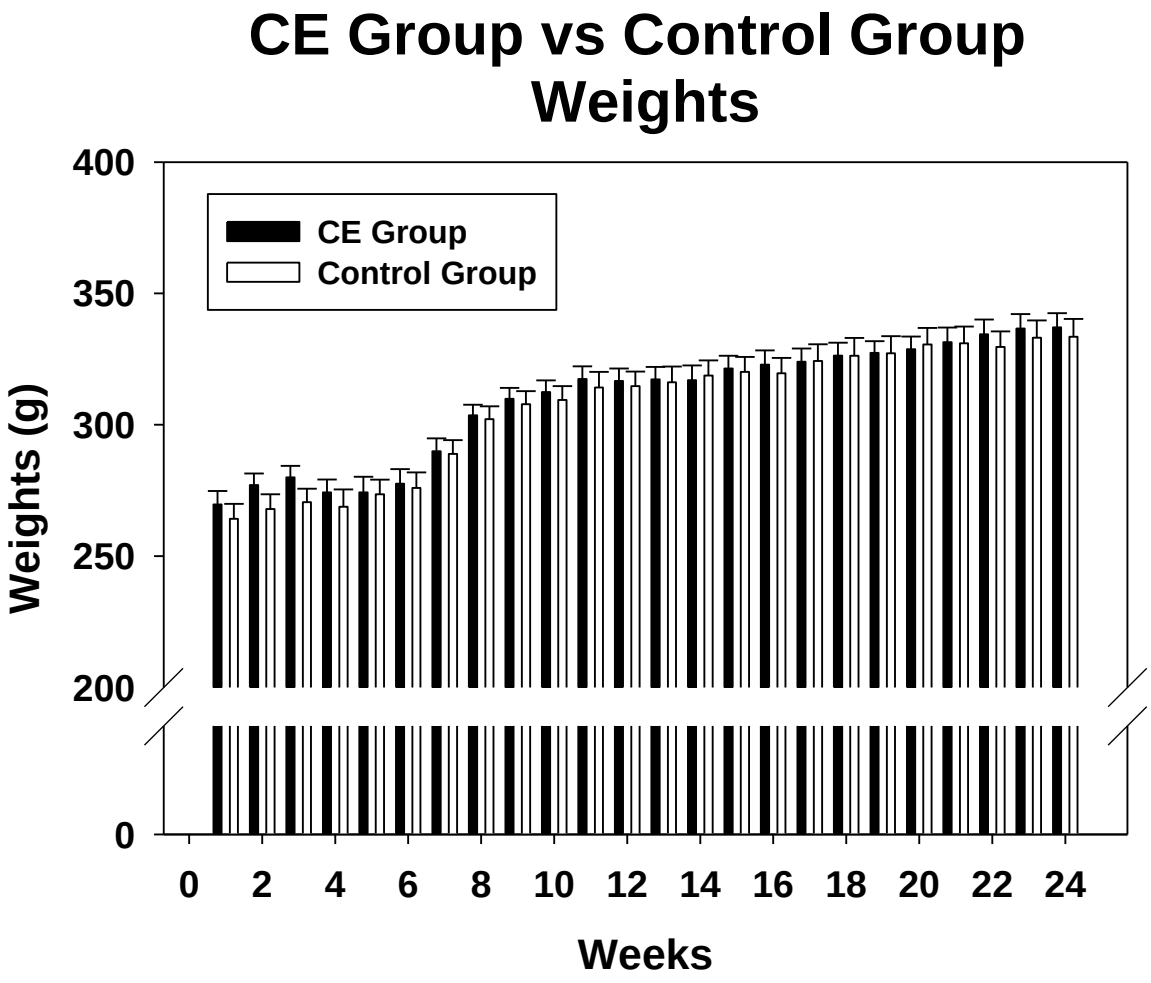


Fig. 2. Weights of CE and control animals (Mean ± SEM body weight in grams) during a 24-week period. Both groups showed steady increases in weight with a significant overall effect due to week but no significant weight difference between groups, or group x week interaction.

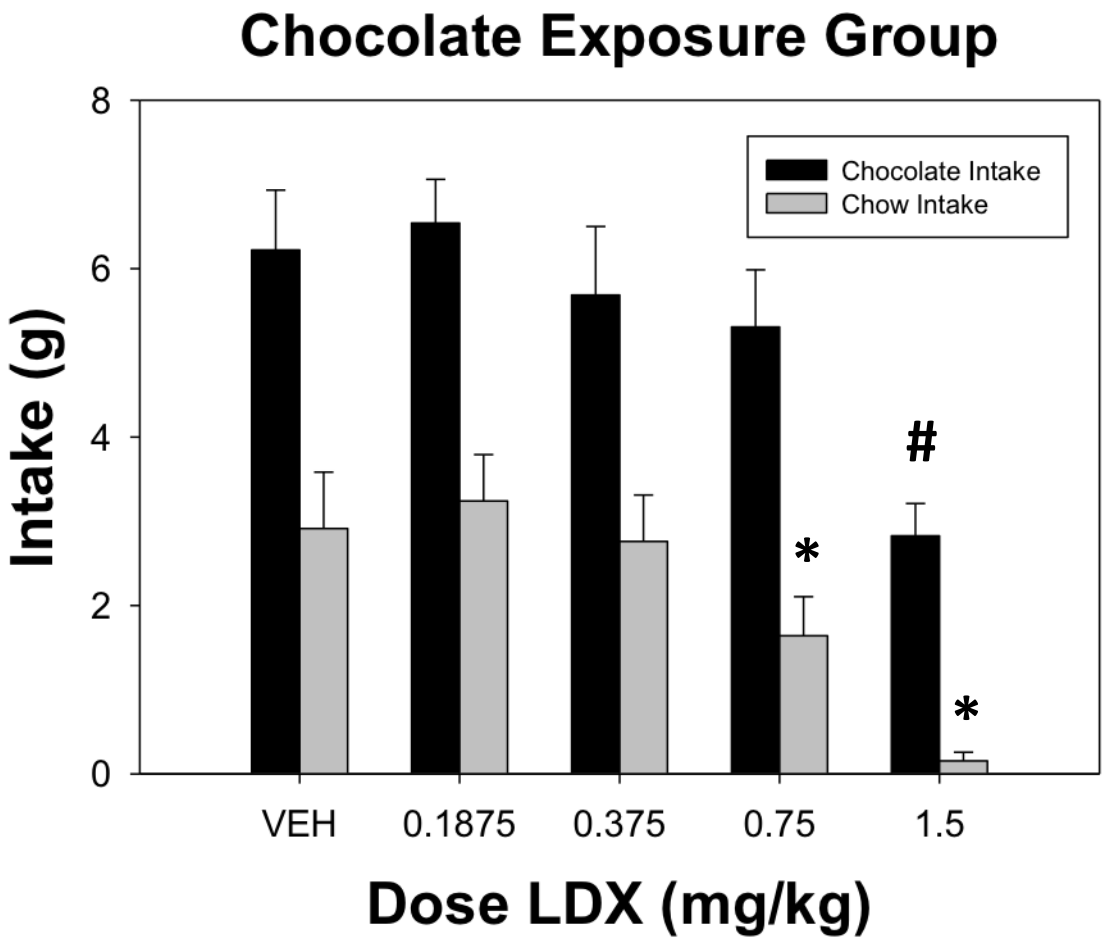


Fig. 3. The effects of LDX on chocolate and chow consumption in the CE group during the 120-minute binge session. Rats (n = 15) received IP injections of vehicle (VEH), 0.1875, 0.375, 0.75, and 1.5 mg/kg doses of LDX. Mean (± SEM) chocolate or chow consumption (in grams) during 120-minute binge session shown. LDX dose significantly different from VEH for chow, *$p < 0.05$; LDX dose significantly different from VEH for chocolate, # $p < 0.05$.

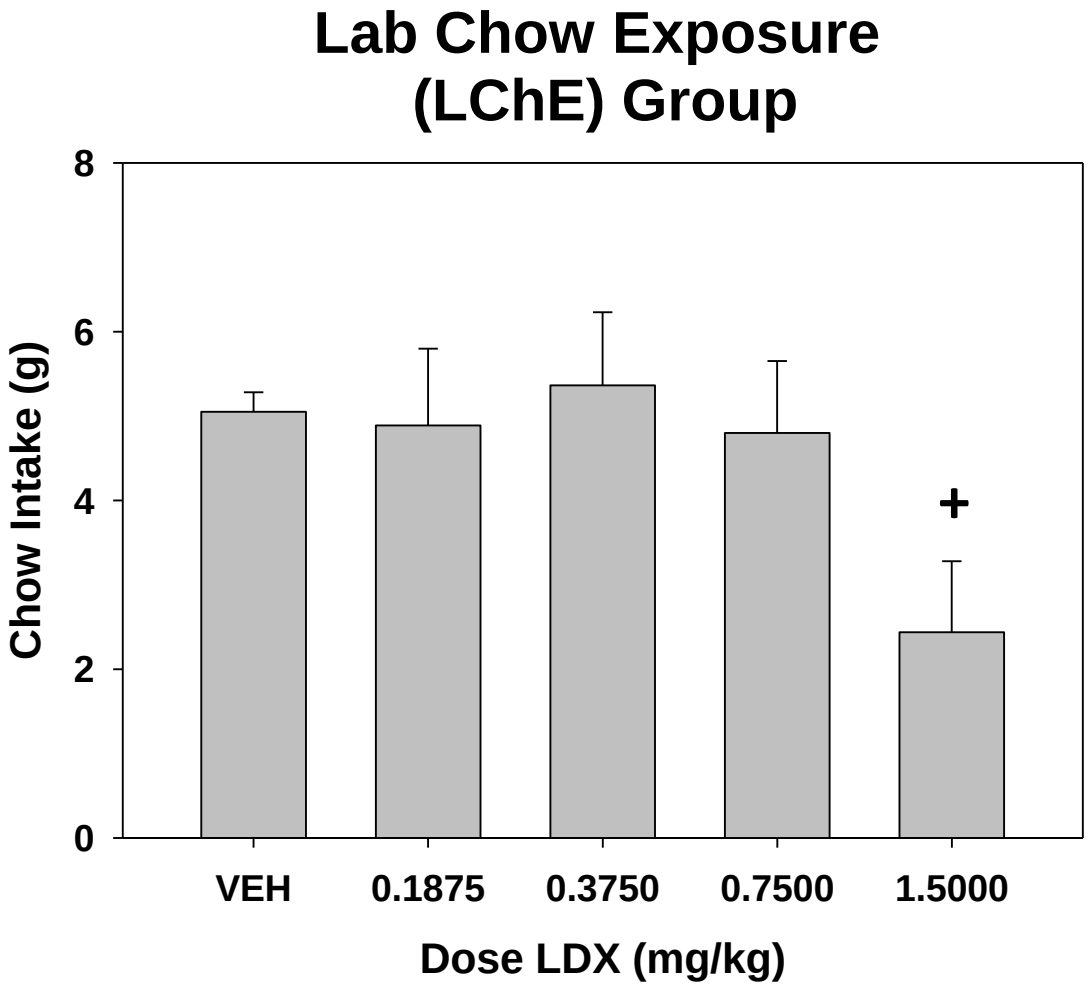


Fig. 4. The effects of LDX on chow consumption in the LChE group during the 120-minute binge session. Rats (n = 8) received IP injections of vehicle (VEH), 0.1875, 0.375, 0.75, and 1.5 mg/kg doses of LDX. Mean (+-SEM) chow consumption (in grams) during 120-minute binge session shown. + LDX dose approached difference from VEH, $0.05 > p < 0.1$.

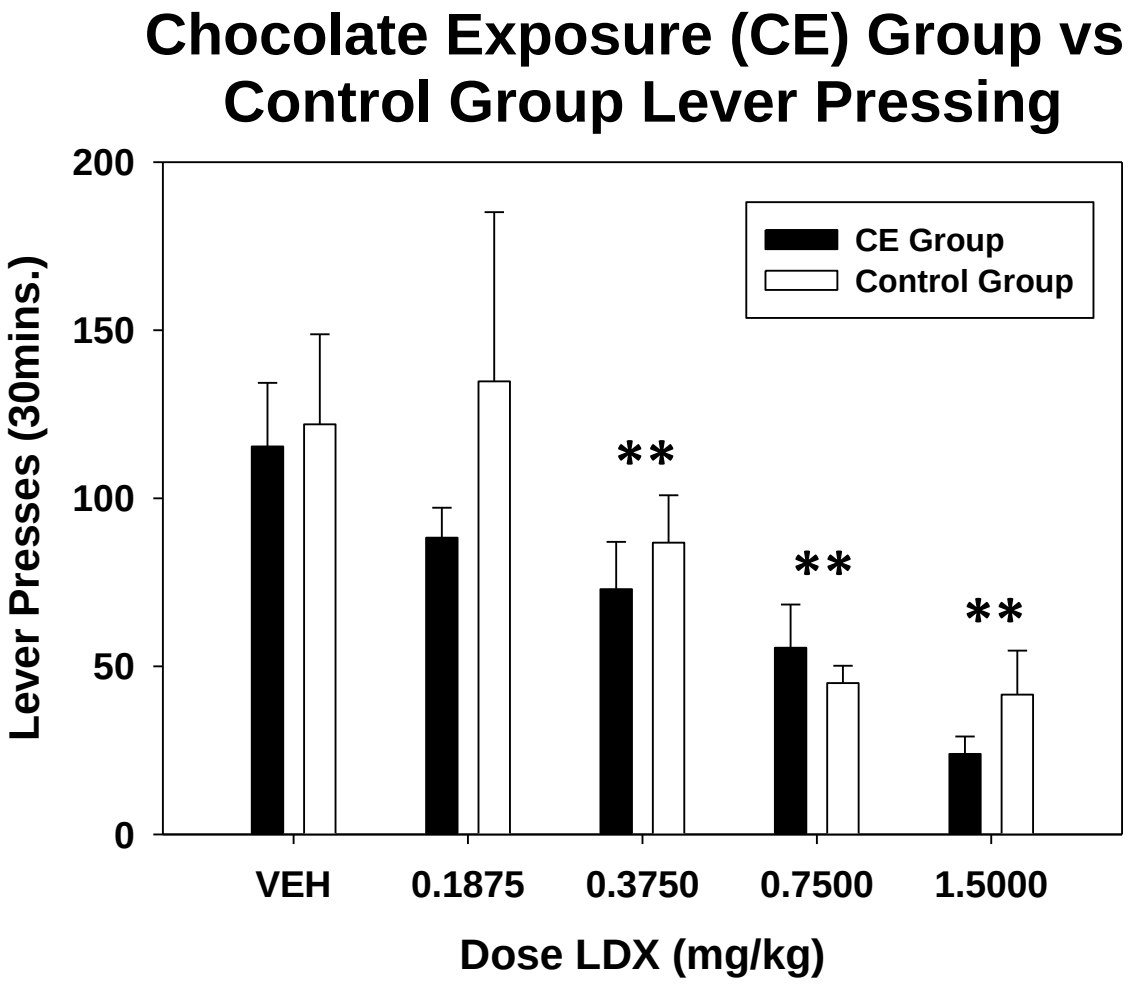


Fig. 5. The effects of LDX on lever pressing in the CE and control groups during the 30-minute operant session. Rats (n = 30) received IP injections of vehicle (VEH), 0.1875, 0.375, 0.75, and 1.5 mg/kg doses of LDX. Mean (± SEM) number of lever presses during 30-minute operant session (PROG/chow choice) shown. LDX dose significantly different from VEH, $^{**}p < 0.001$.

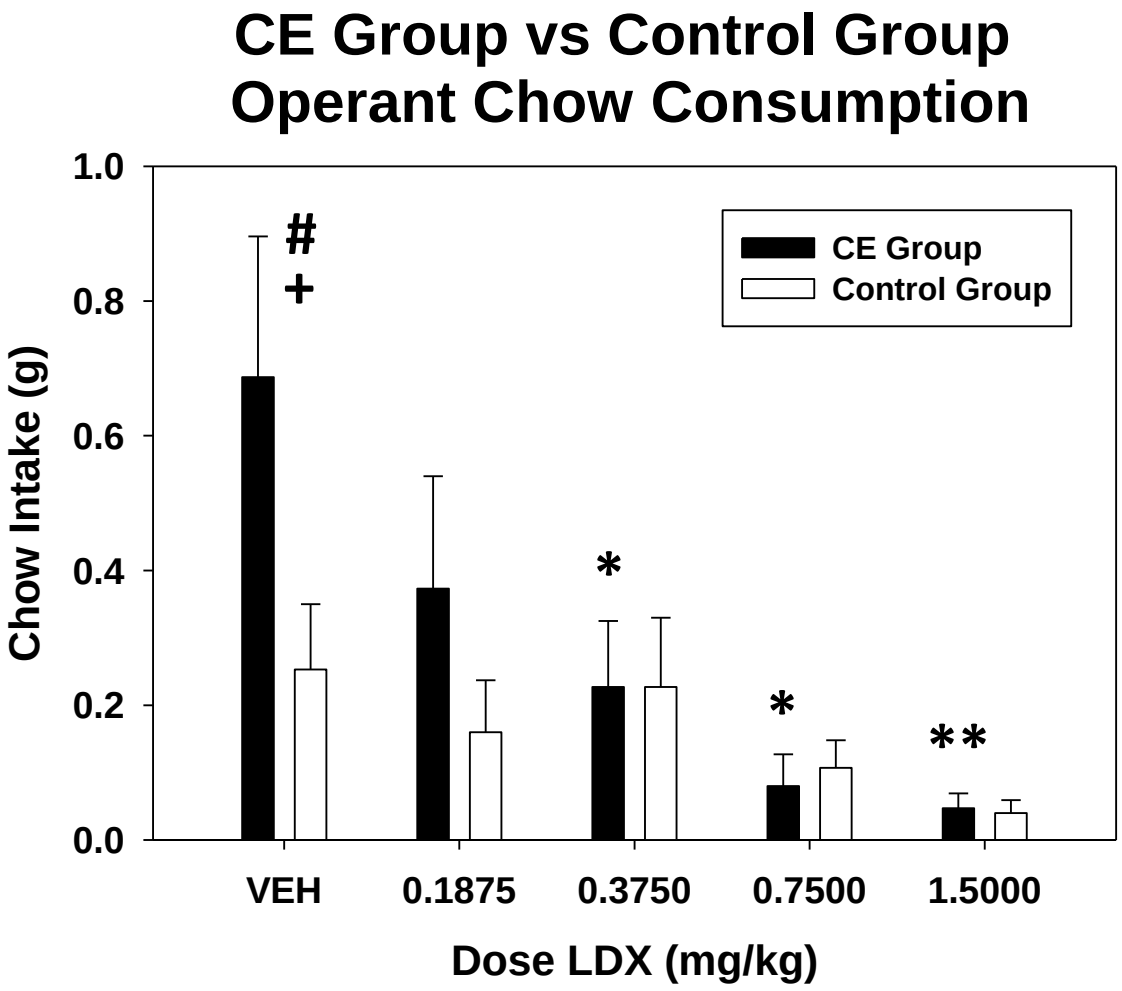


Fig. 6. The effects of LDX on chow consumption in the CE and control groups during the 30-minute operant session. Rats (n = 30) received IP injections of vehicle (VEH), 0.1875, 0.375, 0.75, and 1.5 mg/kg doses of LDX. Mean (± SEM) consumption of chow during 30-minute operant session (PROG/chow choice) shown. LDX dose significantly different from VEH, *$p < 0.05$, **$p < 0.001$. Significant dose by group interaction # $p < 0.05$. Difference between groups +$0.05 < p < 0.1$.

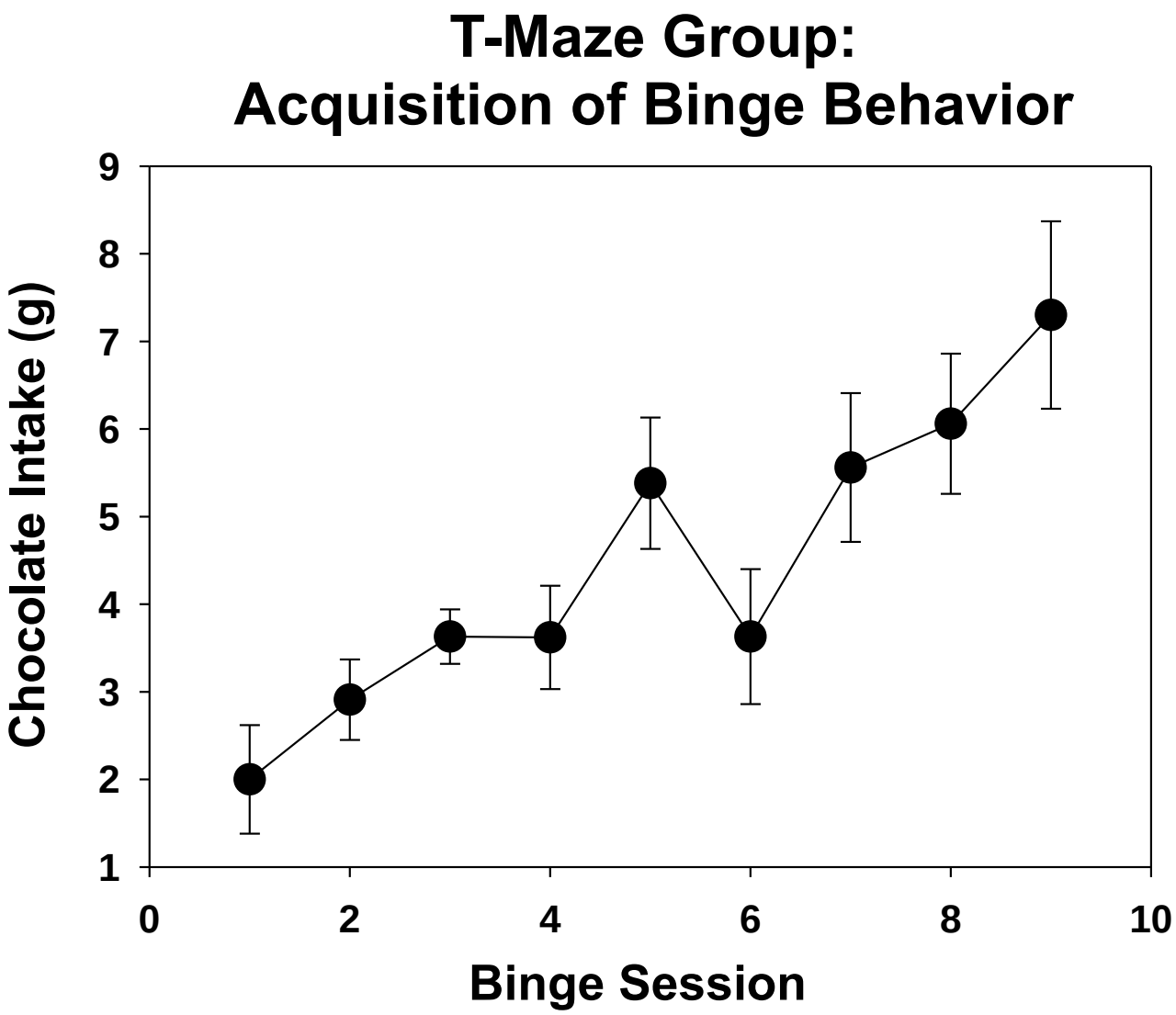


Fig. 7. Chocolate intake for T-Maze group over the course of acquisition of binge eating behavior (Mean ± SEM intake in grams). An increase in chocolate intake was seen over the course of exposure.

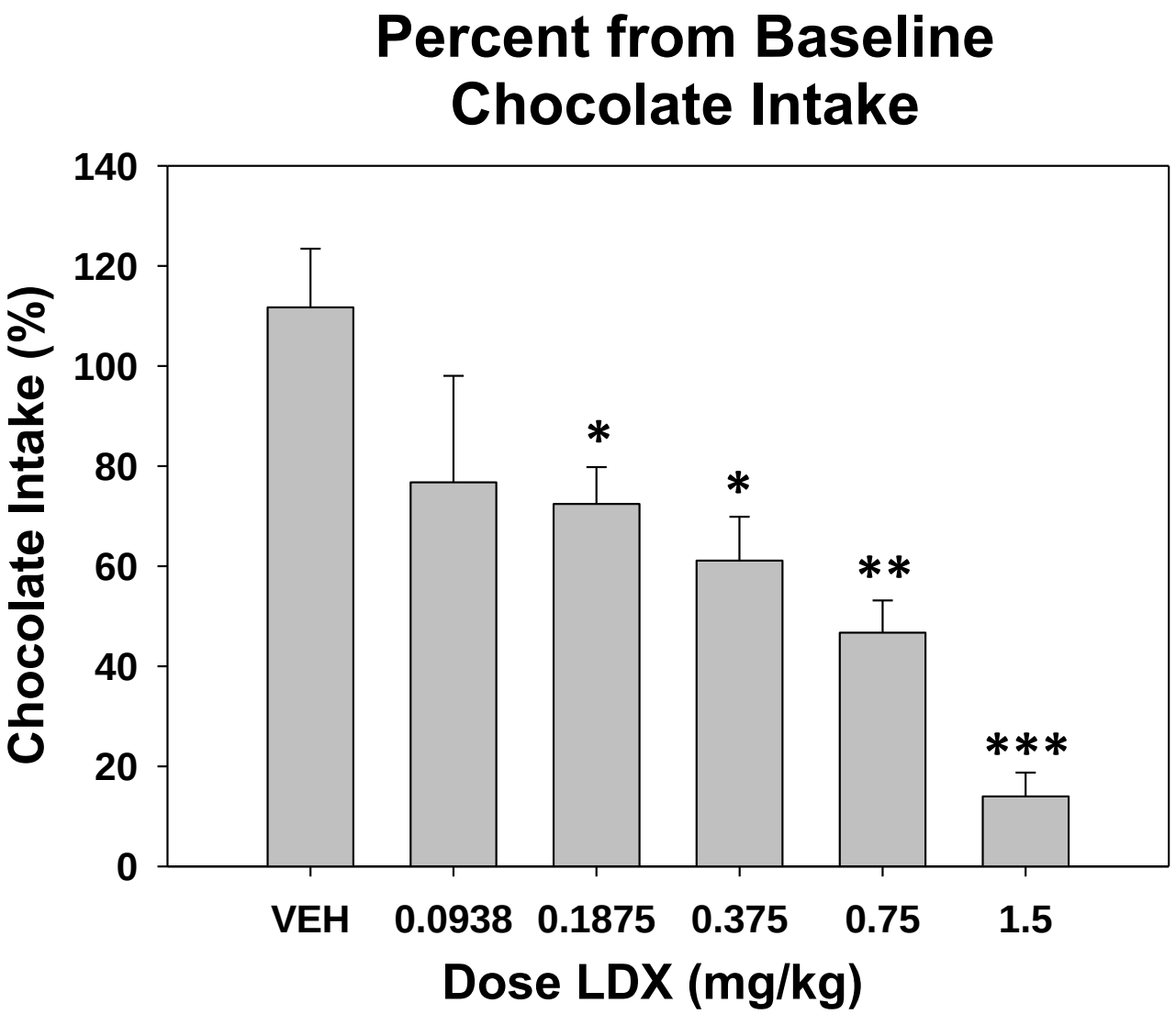


Fig. 8. The effects of LDX on the percentage of averaged 3 day baseline of chocolate consumption. Rats (n = 8) received IP injections of vehicle (VEH), 0.09375, 0.1875, 0.375, 0.75, and 1.5 mg/kg doses of LDX. Mean of percent baseline (± SEM) consumption of chocolate during 30-minute T-Maze session shown. LDX dose significantly different from VEH, *p < 0.05, **p < 0.01, ***p < 0.001.

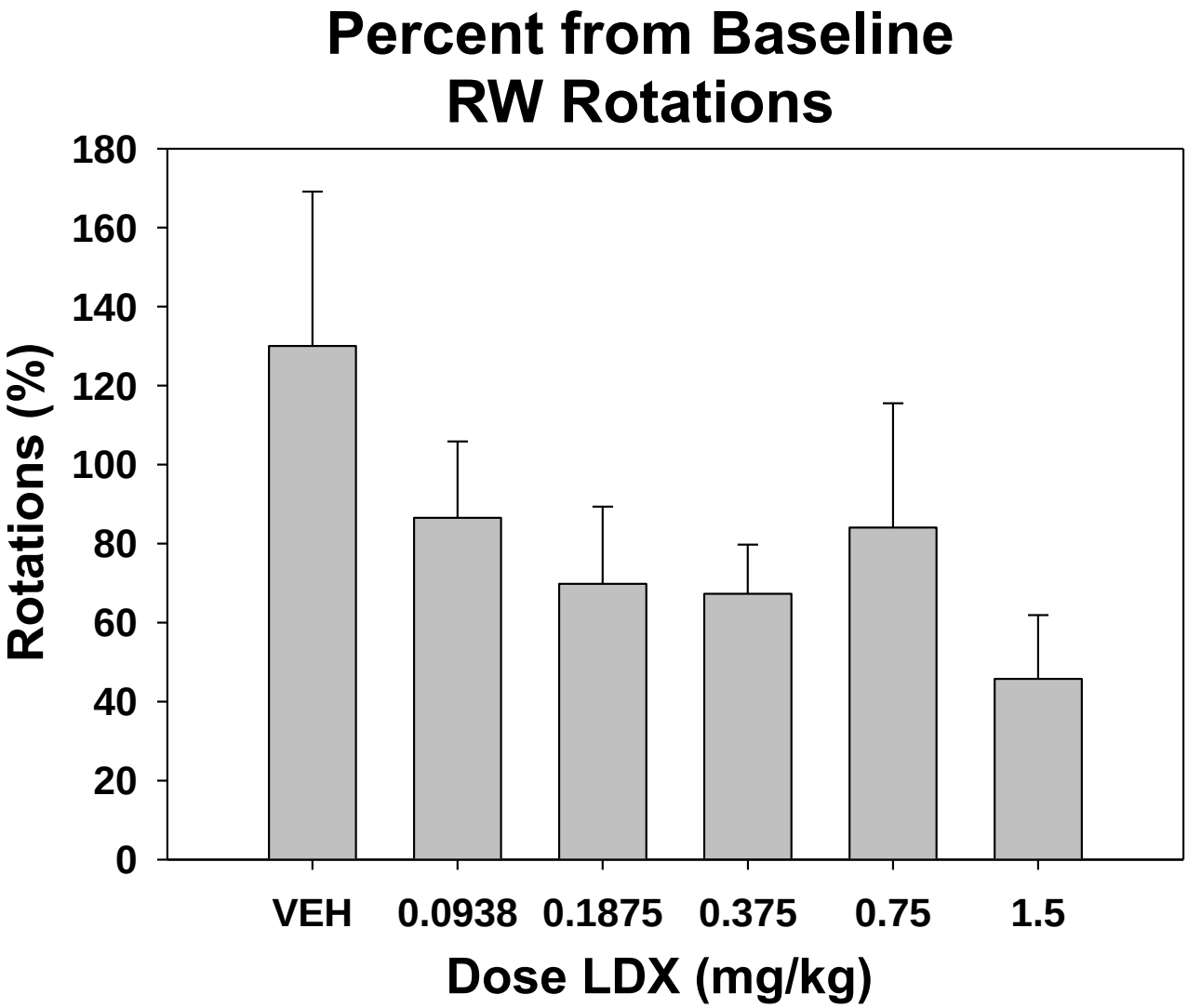
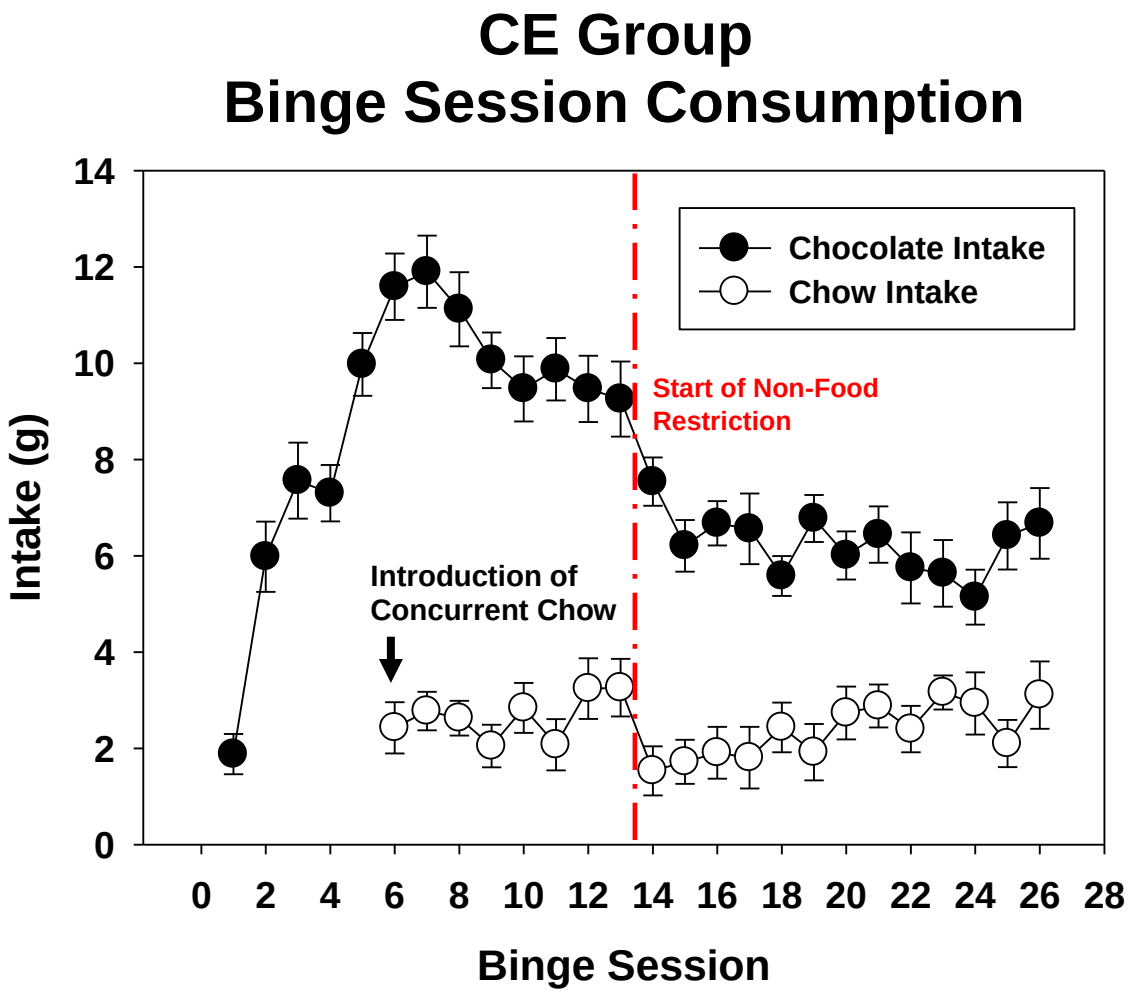
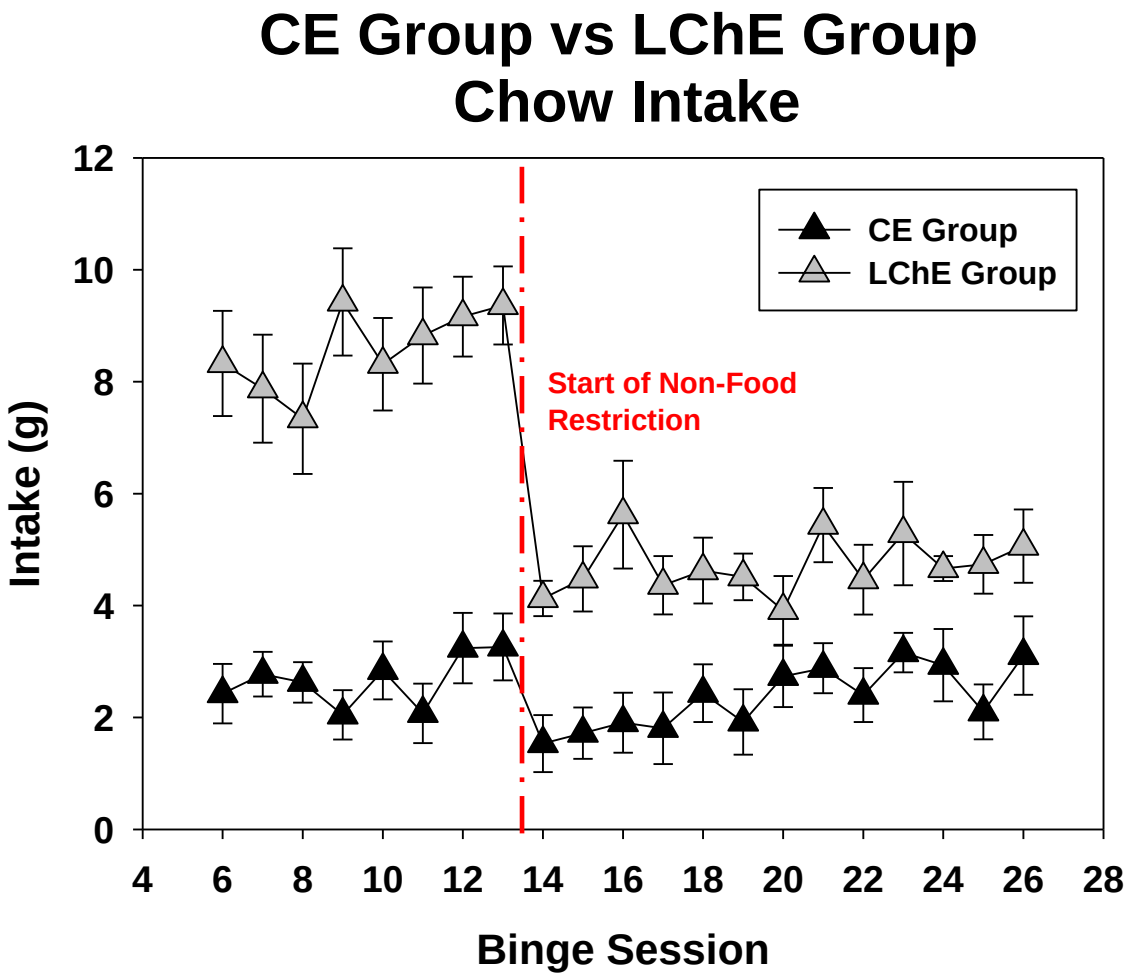


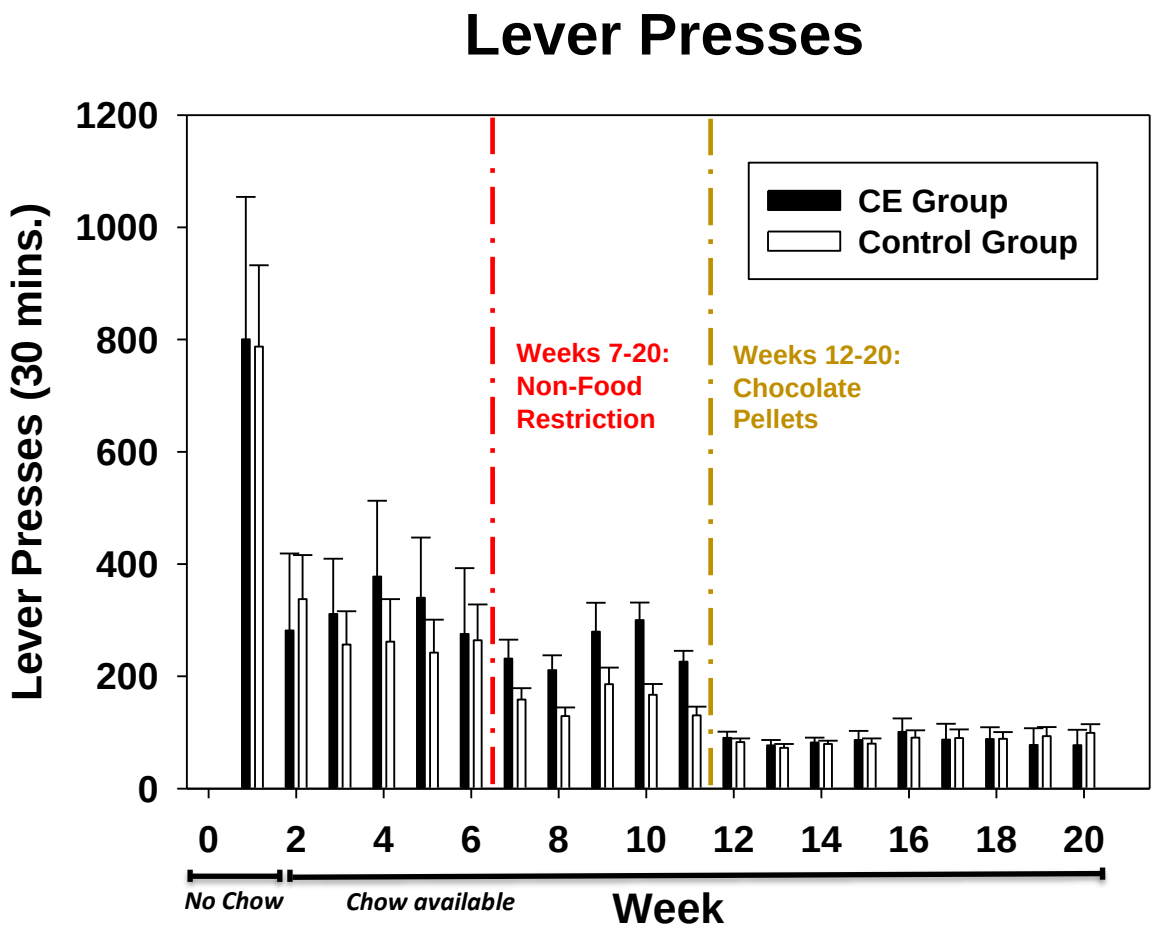
Fig. 9. The effects of LDX on the percentage of averaged 3 day baseline of RW activity. Rats (n = 8) received IP injections of vehicle (VEH), 0.09375, 0.1875, 0.375, 0.75, and 1.5 mg/kg doses of LDX. Mean of percent baseline (± SEM) RW activity determined through RW rotations during 30-minute T-Maze session shown. There was a trend towards a decrease in RW activity by LDX in a dose dependent manner.



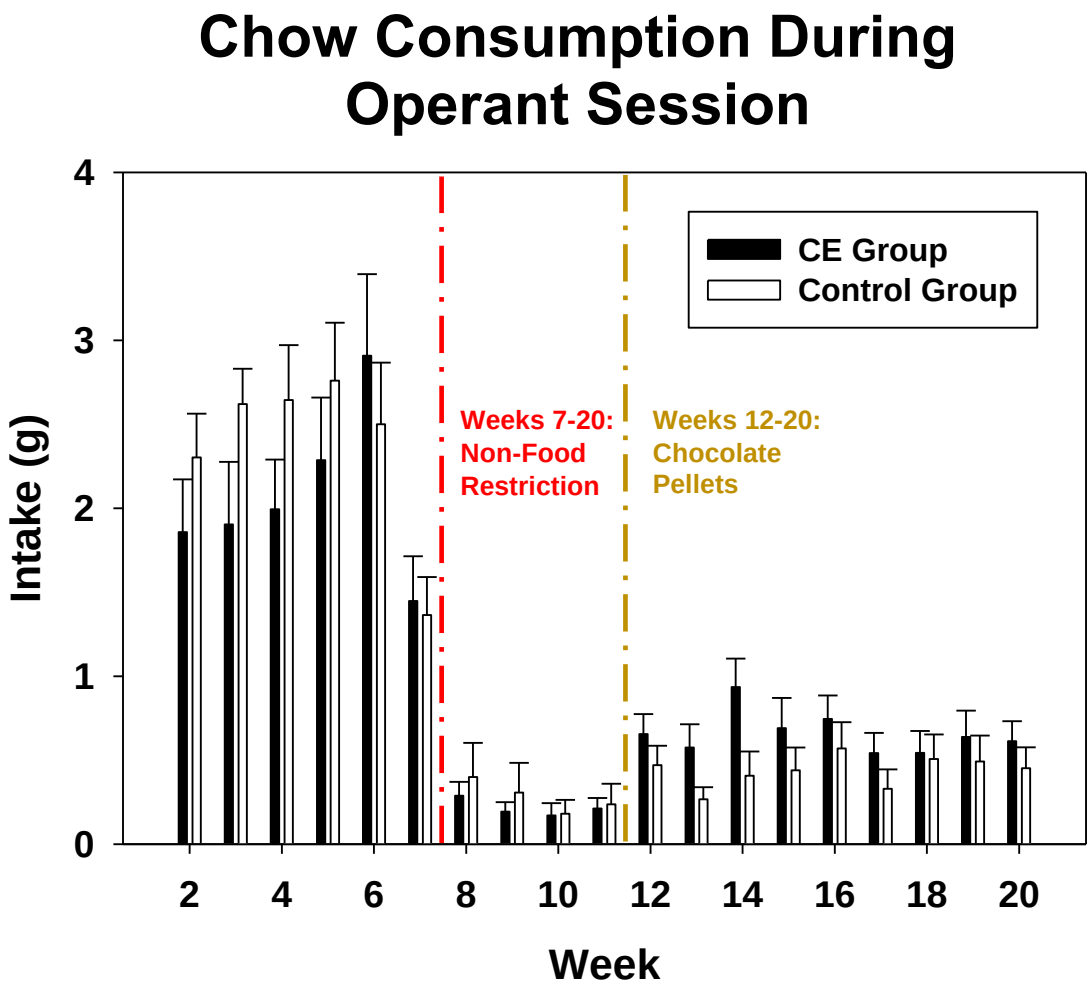
Appendix Fig 1. Chocolate and Chow intakes for CE group over the course of 26 binge sessions. Coinciding with introduction to lab chow during operant task, chow was introduced during 6th binge session. An intake of both foods was reduced once non-food restriction began.



Appendix Fig 2. Chow intake reduced after non-food restriction for both CE and LChE groups during binge eating sessions.



Appendix Fig 3. Weekly average lever pressing for both CE and control groups during 30 minute PROG ratio/feeding task throughout a 20-week period. Groups were food restricted during Weeks 1-6 and non-food restricted Weeks 7-20. Concurrently available lab chow was introduced at the start of Week 2. Reward was plain high carbohydrate pellets weeks 1-11 and chocolate flavored weeks 12-20.



Appendix Fig 4. Chow intake during operant session for both CE and control groups during 30 minute PROG ratio/feeding task throughout a 20-week period. Groups were food restricted during Weeks 1-6 and non-food restricted Weeks 7-20. Shifting from food restriction to non-food restriction decreased chow intake in both groups.

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