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The Effects of Activity-Based Anorexia on the Rewarding Properties of Methamphetamine and Wheel Running

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Abstract

Anorexia nervosa is the third most common chronic illness among adolescents, with an estimated prevalence of 1% in females. Research has shown that up to 80% of individuals with anorexia nervosa engage in excessive exercise, leading some researchers to propose that exercise may have addiction-like properties in people with this disorder. Addiction to drugs of abuse has also been linked to eating disorders, with a lifetime comorbidity of roughly 20%. While previous studies have used rodent models to understand the association between food restriction and the rewarding effects of drugs of abuse, it is not known if the addition of exercise changes these effects. We used activity-based anorexia (ABA), a widely used rodent model of anorexia that combines food restriction and physical activity, to further explore whether anorexia during adolescence affects circuits underlying reward. This involved testing the effects of ABA on the rewarding properties of methamphetamine (1mg/kg) and wheel running in two different strains of female mice, C57Bl/6 and 129/SvEv. We found that methamphetamine (1mg/kg, i.p.) induced conditioned place preference in adolescent female C57Bl/6 mice but not 129/SvEv mice. The ABA paradigm significantly enhanced methamphetamine-induced conditioned place preference in the C57Bl/6 strain. Additionally, we found no effect of ABA on the rewarding effects of wheel running in either strain, as measured by a modification of conditioned place preference procedures. These results indicate that there is a strain difference in the rewarding effects of methamphetamine in adolescent female mice, and that experience in the ABA paradigm enhances the rewarding properties of methamphetamine, but not wheel running. Additional experiments involving larger groups of animals and an examination of individual differences are required to further understand the role of wheel running in ABA.

The Effects of Activity-Based Anorexia on the Rewarding Properties of Methamphetamine and Wheel Running

Anorexia nervosa is a psychiatric illness characterized by extreme restriction of food intake, an irrational fear of gaining weight, and an inappropriate assessment of body size (Kaye et al., 2013). The onset of anorexia often occurs around mid-adolescence and is the third most common chronic illness found in this age group (Whitaker, 1982), with an estimated prevalence of 1% in females (National Institute of Mental Health, 2018). Despite having the highest lifetime mortality rate of all psychiatric illnesses in young females (10%), few effective treatment strategies exist today (Birmingham et al., 2005).

Although not part of the formal diagnostic criteria, excessive physical activity is commonly seen in individuals with anorexia nervosa, with 31-81% of patients exhibiting high activity levels (Hebebrand et al., 2003). Although this typically manifests as compulsive or compensatory voluntary exercise, increased non-exercise activity, such as fidgeting, has also been observed (Kron et al., 1978). Moreover, excessive exercise is associated with poorer outcomes (Strober et al., 1997), including greater risk of relapse, longer hospitalizations, and increased duration of disease, indicating that it may play a role in maintenance of the disorder. Physical activity may also contribute to the development of anorexia, an effect found even among athletes (Davis et al., 1994). The observation that individuals with anorexia commonly engage in excessive exercise (i.e., more than one hour a day for at least six days a week, for a period of one month or more), has led some to propose that exercise may have addiction-like properties in people with this disorder (Davis et al., 2002).

Addiction to drugs of abuse has also been linked to eating disorders (Bahji et al., 2019; O'Brien et al., 2003). Roughly 20% of individuals diagnosed with an eating disorder (i.e., anorexia nervosa, bulimia nervosa, and binge eating disorder) develop a comorbid substance use disorder, with lifetime comorbidity being higher among females (Bahji et al., 2019). Conversely, women seeking treatment for substance abuse disorders also have a high rate of eating disorders (41%) (Grilo et al., 1997). Consistent with this work, animal studies have shown that food restriction in rats increases the rewarding effects of cocaine (Liu et al., 2011; Zheng et al., 2013; Zheng et al., 2011), which may be mediated by enhanced expression of AMPA receptors in the nucleus accumbens (NAc) (Zheng et al., 2015). Together, this indicates that food restriction may influence reward circuits. However, it is not known whether compulsive exercise in combination with food restriction has similar effects.

Activity-based anorexia (ABA) is a widely used rodent model of anorexia nervosa that involves giving food restricted animals unlimited access to a running wheel. Under these conditions, rodents exhibit hyperactivity, self-starvation, rapid weight loss, and death unless removed from the experiment. Given that individuals with anorexia commonly engage in compulsive exercise, this model may capture features of anorexia beyond food restriction alone. In the present study, we tested whether anorexia during adolescence affects the development of reward circuits by testing the effects of adolescent ABA on the rewarding properties of methamphetamine and wheel running in female mice. It is hoped that this work leads to a better understanding of neural circuits underlying anorexia nervosa, potentially leading to the development of novel treatments.

Experiment 1: ***Piloting methamphetamine-induced conditioned place preference (CPP) in two strains of female mice***

Introduction

I first established a protocol for measuring the rewarding properties of methamphetamine using the conditioned place preference paradigm (CPP). CPP has been used extensively to measure the reinforcing effects of drugs of addiction in rodents (Huston et al., 2013) and typically involves associating a drug of abuse with a particular compartment of the apparatus. An increase in the amount of time spent in the compartment previously paired with a drug is considered to reflect the rewarding properties of that drug (Huston et al., 2013). We tested the rewarding effects of methamphetamine because of its clinical relevance in substance abuse disorders (Gonzales et al., 2010), its use in females for weight loss (Brecht et al., 2004), and its previous use to induce CPP in male rodents (Der-Ghazarian et al., 2019; Taslimi et al., 2018).

Method

Animals

I tested two strains of mice that are commonly used as background strains for transgenic lines. Female C57Bl/6 and 129/SvEv mice (Taconic Biosciences, Germantown, NY) were shipped to the Hunter College Animal Facility at postnatal day (PND) 21. Mice were group-housed 4 per cage and kept on a 5am/5pm, 12-h light/dark cycle with food and water available *ad libitum*. Experimental procedures began on PND 38 (middle adolescence) and all testing occurred during a portion of the light cycle between 10am and 4pm. Experiments were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) of Hunter College, CUNY and the NIH Guide for the Care and Use of Laboratory Animals.

Apparatus

Conditioned place preference was conducted using a three-compartment apparatus (Columbus Instruments, Columbus, OH), which consisted of one light side, one dark side and a holding chamber. The light side was entirely white Plexiglas and scented with orange Clorox wipes. The dark side had black Plexiglas walls with a textured red and white striped floor scented with 100% ethanol. A removable divider was used to separate the light and dark compartments during conditioning sessions. The holding chamber protruded from the side and was equipped with an adjoining door, providing access to both the light and dark compartments. Prior to each session, the holding chamber was wiped down with a wet paper towel and the light and dark chambers were wiped down with their respective scents. The light and dark compartments were each 8.25” long, 12” high and 12” wide. The holding chamber was 3.5” long x 3.5” wide x 5” deep.

Drugs

Methamphetamine hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in sterile saline (0.9%). On each day of drug administration, methamphetamine was prepared fresh prior to being injected intraperitoneally (i.p.) at a dose of 1mg/kg.

Procedure

The 12-day CPP procedure consisted of four phases: handling (days 1 and 2), preconditioning (day 3), conditioning (days 4-11), and postconditioning (day 12). At the start of each session, all mice were weighed and given a distinct tail marking with a Sharpie pen for

identification. Handling occurred on PNDs 38 and 39, and involved holding each mouse by the tail for 2 minutes while the mouse walked freely along the experimenter's gloved hand and sleeve. During preconditioning (PND 40), each mouse was allowed to freely explore both light and dark compartments for a total of 30 minutes. Following preconditioning, initial baseline preference was assessed, and the drug was randomly assigned to either the light or dark side throughout conditioning sessions (unbiased design). Conditioning then took place on the subsequent 8 days (PND 41-48), with mice in the drug group receiving drug every other day. On days 4, 6, 8, and 10, mice received either methamphetamine (drug group) or saline (saline group) before being confined to one side for 30 minutes. On days 5, 7, 9, and 11, mice in both groups were injected with saline prior to being confined to the other side for 30 minutes. During the postconditioning test (PND 49), mice had free access to the light and dark compartments for 30 minutes and time spent in each was measured. Animals were not injected with drug or saline during the postconditioning test. Cameras mounted above the conditioned place preference apparatus recorded behavior throughout preconditioning and postconditioning sessions.

Statistical Analysis

Videos were analyzed using ANY-maze software (Stoelting, Wood Dale, IL) to determine time spent on both sides of the conditioned place preference box. Preference was determined by calculating the difference in time spent in the drug-paired compartment before and after conditioning. Data were analyzed with a two-way ANOVA (GraphPad Prism, San Diego, CA) and Tukey's HSD was used for post-hoc analyses.

Results

C57Bl/6 mice

A two-way ANOVA on time spent in the drug-paired side revealed a significant main effect of time spent (Pre-CPP vs Post-CPP) ($F(1,30) = 4.659$, $p < 0.05$), but no significant main effect of drug ($F(1,30) = 0.040$, $p = 0.84$) and a drug × time spent interaction that approached significance ($F(1, 30) = 4.109$, $p = 0.052$). Post-hoc tests showed that the C57Bl/6 mice in the methamphetamine group spent significantly more time in the drug-paired side compared to the C57Bl/6 saline group ($p < 0.05$). Additionally, there was no significant difference between time spent on the drug-paired side during preconditioning for both groups ($p > 0.05$). These results indicate that methamphetamine-induced conditioned place preference in adolescent female C57Bl/6 mice (Figure 1).

129/SvEv mice

Time spent on the drug-paired side during preconditioning and postconditioning was assessed in 129/SvEv mice. A two-way ANOVA revealed no significant main effect of time spent (Pre-CPP vs Post-CPP) ($F(1,30) = 0.965$, $p = 0.336$), drug ($F(1,30) = 0.022$, $p = 0.884$) or drug × time spent interaction ($F(1, 30) = 0.847$, $p = 0.365$). Additionally, there was no significant difference between time spent on the drug-paired side during preconditioning for both groups ($p > 0.05$). These results indicate that unlike C57Bl/6 mice, 129/SvEv mice are less sensitive to the rewarding effects of methamphetamine (Figure 2).

Experiment 2: *An investigation of the effects of ABA on methamphetamine-induced CPP*

Introduction

There is mounting evidence from neuroimaging studies demonstrating that reward processing is altered in anorexia nervosa. It has been proposed that this leads to anhedonia, which may contribute to the development and maintenance of anorexia nervosa (Foldi et al., 2017b). Similarly, studies suggest a strong association between anhedonia and substance abuse (Destoop et al., 2019; Garfield et al., 2014), the latter of which is commonly found in individuals previously diagnosed with an eating disorder (Bahji et al., 2019; O'Brien & Vincent, 2003). Together, these findings suggest that anorexia nervosa during adolescence may alter the development of reward circuits. The aim of this second experiment was to test this idea by testing whether ABA affects subsequent responses to methamphetamine in the conditioned-place preference paradigm.

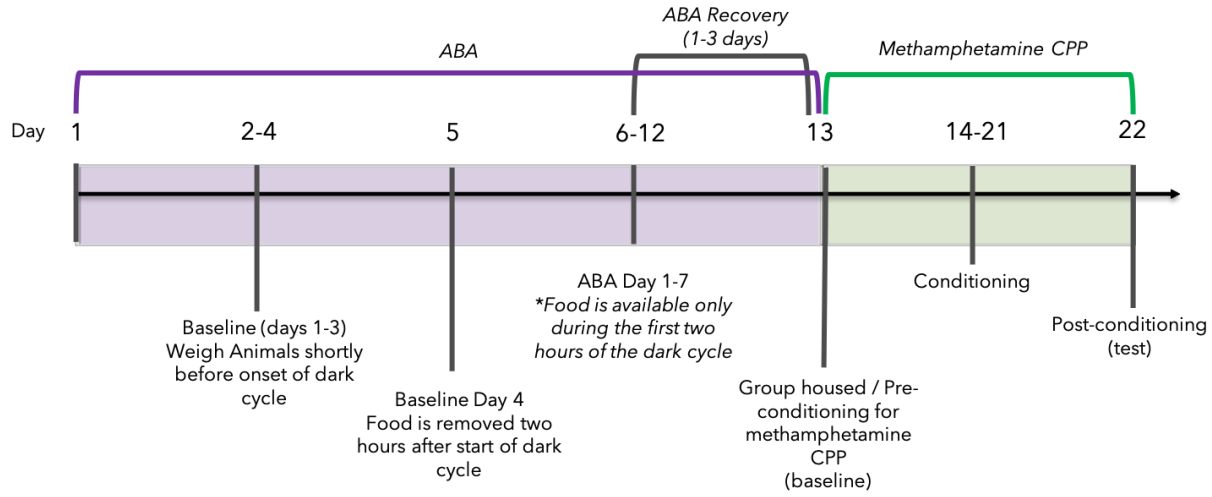
Based on the results from our pilot experiment, we opted to use the C57/Bl6 strain in this experiment, as they appear to be more sensitive to methamphetamine-induced CPP than the 129/SvEv strain. Furthermore, previous work in our lab has shown that this strain is also more vulnerable to activity-based anorexia than the 129s.

Method

Animals and General Procedures

Female C57Bl/6 mice (Taconic Biosciences, Germantown, NY) were shipped to the Hunter College Animal Facility at postnatal day (PND) 21. Initially, mice were group-housed 4 per cage and kept on a 5am/5pm, 12-h light/dark cycle with food and water available *ad libitum*. Roughly 2.5 weeks later, at (PND) 38, animals were individually housed with either a running

wheel (ABA group) or a locked wheel (Home Cage control group) (wireless wheels, Med Associates, VT) for the ABA phase of the experiment (see below). CPP began immediately following 1-3 days of recovery from ABA and occurred during the light cycle (10am and 4pm) (see timeline below). Experimental procedures were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) of Hunter College, CUNY and in accordance with the NIH Guide for the Care and Use of Laboratory Animals.



ABA Procedures

Mice were individually housed on PND 38 and left untouched the next day, during which time they acclimated to their new living conditions (locked wheel for HC; freely moving wheel for ABA). The following day was the first of 4 baseline days (baseline days 1-4). On each baseline day, mice, their water and their food pellets were weighed immediately prior to the onset of the dark cycle (5:00 p.m.). On Baseline Day 4, food was removed from mice in the ABA group two hours after the onset of the dark cycle (7:00 p.m.) and water remained available *ad libitum*. The next day (ABA Day 1), mice were weighed immediately prior to the onset of the dark cycle and mice in the ABA group were given an unlimited amount of food for the first 2 hours of the dark cycle (5pm-7pm). This was repeated for 7 days total (ABA Days 1-7) or until

animals met criteria for removal from the experiment (more than 25% loss of baseline body weight). Once criteria was met, mice were removed from ABA, given unlimited access to food, and allowed to recover. Recovery time ranged from 1-3 days before CPP began. Mice in the HC group were treated the same way, except food was never removed. On Day 8, all animals (PND 53) were group housed and baseline preconditioning for CPP was assessed.

Methamphetamine-CPP

The 10-day CPP procedure consisted of three phases: preconditioning (day 1), conditioning (days 2-9), and postconditioning (day 10). At the start of each session, all mice were weighed and given a distinct tail marking with a Sharpie pen for identification purposes. During preconditioning (PND 50), each mouse was allowed to freely explore the light and dark compartments for a total of 30 minutes. Following preconditioning, initial baseline preference was assessed, and the drug was subsequently paired on the least preferred side throughout conditioning sessions (biased design). Conditioning then took place on the subsequent 8 days (PND 51-58). On days 2, 4, 6, and 8, all mice in both the ABA and HC groups received methamphetamine before being confined to the least preferred compartment for 30 minutes. On days 3, 5, 7, and 9, all mice in both groups were injected with saline prior to being confined to the preferred side for 30 minutes. During the postconditioning test (PND 59), all mice had free access to the light and dark compartments for 30 minutes and preference was tested. Animals were not injected with drug or saline during the postconditioning test. Cameras mounted above the conditioned place preference apparatus recorded behavior throughout preconditioning and postconditioning sessions.

Statistical Analysis

Videos were analyzed using ANY-maze software (Stoelting, Wood Dale, IL) to determine time spent on each side of the conditioned place preference box. Preference was determined by calculating the difference between the amount of time spent in the drug-paired compartment before and after conditioning. A two-way ANOVA was used to compare the amount of time each group spent in the drug-paired side before and after conditioning and Tukey's HSD was used for post-hoc analyses.

Results

C57Bl/6 mice

Survival curve data indicating the rate of removal from the ABA model, average percent body weight on the day of ABA removal, and average percent body weight on the first day of methamphetamine conditioning can be found in Figure 3. Time spent on the drug-paired side during preconditioning and postconditioning was assessed in the ABA and HC groups. A two-way ANOVA revealed a significant main effect of time spent (Pre-CPP vs Post-CPP) ($F(1,5) = 65.03$, $p < 0.01$) and a significant HC vs ABA x time spent interaction ($F(1,5) = 23.99$, $p < 0.01$), but no significant main effect of HC vs ABA ($F(1,5) = 0.260$, $p = 0.632$). Post-hoc tests showed that C57Bl/6 mice in the ABA group spent significantly more time on the drug-paired side postconditioning compared to preconditioning ($p < 0.01$). These results indicate that mice in the ABA group are more sensitive to the methamphetamine-induced conditioned place preference paradigm compared to the HC group (Figure 4).

Experiment 3: *An investigation of the effects of ABA on the rewarding effects of wheel running*

Introduction

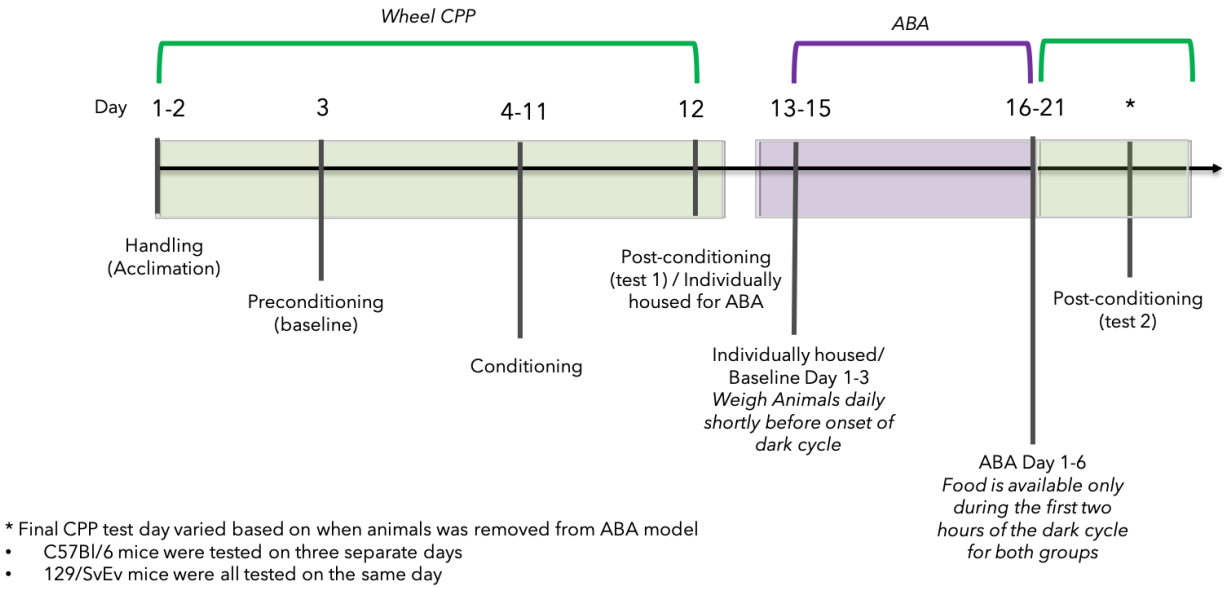
The observation that individuals with anorexia commonly engage in excessive exercise has led some to propose that exercise may have addiction-like properties in people with this disorder (Davis et al., 2002). This idea is supported by the finding that food restriction increases the rewarding effects of appetitive stimuli (Carr, 2011; Peng et al., 2014; Jung et al., 2016; Zheng et al., 2015). To test whether hyperactivity during ABA reflects an increase in the rewarding properties of wheel running, we modified the conditioned place preference (CPP) procedure such that one compartment was associated with a running wheel. To test whether mice in the ABA model prefer running over eating, we paired the other compartment with food. Mice of both strains were tested in this Wheel-CPP paradigm following exposure to ABA or food restriction alone.

Method

Animals

Female C57Bl/6 and 129/SvEv mice (Taconic Biosciences, Germantown, NY) were shipped to the Hunter College Animal Facility at postnatal day (PND) 21. Initially, mice were group-housed 4 per cage and kept on a 5am/5pm, 12-h light/dark cycle with food and water available *ad libitum*. Training in the wheel-CPP procedure began on PND 38 (middle adolescence) during the light cycle (10am and 4pm). Mice were individually housed for ABA immediately following the first postconditioning test. Mice were tested in a final postconditioning test after losing a substantial amount of weight and while they were still hungry

(no recovery) (see timeline below). All experimental procedures were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) of Hunter College, CUNY and in accordance with the NIH Guide for the Care and Use of Laboratory Animals.



Wheel-CPP

The 13-day CPP procedure consisted of five phases: handling (days 1 and 2), preconditioning (day 3), conditioning (days 4- 11), postconditioning 1 (day 12) and postconditioning 2 (day 13). At the start of each session, all mice were weighed and given distinct tail marking with a Sharpie pen for identification purposes. Handling occurred on PND 38 and 39 and involved gently holding the mouse by the tail for 2 minutes while it walked freely on the experimenter's gloved hand and sleeve. During preconditioning (PND 40), each mouse was allowed to freely explore the light and dark compartments for 30 minutes. Conditioning took place on the subsequent 8 days (PND 41-48). During conditioning, one compartment always contained a weight boat with food pellets and one compartment always contained a running wheel. For half of the animals in each group, the running wheel was associated with the dark compartment and for the other half, it was associated with the light compartment. During

conditioning sessions, all animals were confined to either compartment for 30 minutes. During postconditioning test 1 (PND 49) and postconditioning test 2 all mice had free access to the light and dark compartments for 30 minutes and preference was tested. Cameras mounted above the conditioned place preference apparatus recorded behavior throughout preconditioning and both postconditioning sessions. Animals were individually housed immediately following the first post-conditioning session and allocated to either the ABA or food restriction control (FR) group.

ABA/Food Restriction

Mice were individually housed on PND 49. Those in the ABA group were housed with freely moving wheels, while those in the food restriction control group (FR) were housed with locked wheels. The following day was the first of 3 baseline days (baseline days 1-3). On each baseline day, mice, their water and their food pellets were weighed immediately prior to the onset of the dark cycle (5:00 p.m.). On Baseline Day 3, food was removed from both groups two hours after the onset of the dark cycle (7:00 p.m.) and water remained available *ad libitum*. The next day (ABA Day 1), mice were weighed immediately prior to the onset of the dark cycle and given unlimited access to food during the first 2 hours of the dark cycle (5pm-7pm). This was repeated for a maximum of 6 days total (ABA Days 1-6). ABA testing ended earlier for mice that were expected to lose 25% of their baseline body weight within 24 hours. The morning after the last night of food restriction, both groups were reintroduced to the wheel-CPP apparatus for a final postconditioning test while hungry (no recovery). In this experiment, individual C57Bl/6 mice were tested in the final postconditioning test on three separate days, as they were removed from ABA at different times. 129/SvEv mice, which are less vulnerable to ABA, were all tested in the final postconditioning test on the same day.

Statistical Analysis

Videos were analyzed using ANY-maze software (Stoelting, Wood Dale, IL) to determine time spent on each side of the conditioned place preference box. Wheel preference was determined by comparing the time spent in the wheel-paired compartment during postconditioning with the amount of time spent in that same compartment during preconditioning. Additionally, food preference was determined by comparing the time spent in the food-paired compartment during postconditioning with the amount of time spent in that same compartment during preconditioning.. A two-way ANOVA was used to analyze the time spent on the wheel-paired and food-paired side. Tukey HSD was used for post-hoc analyses.

Results

C57Bl/6 mice

Survival curves showing removal of each group from the ABA paradigm, along with the average percent baseline body weight on the final postconditioning (test 2) day for both ABA and FR groups, can be found in Figure 5. There was no significant difference between the ABA and FR groups in average percent baseline body weight on the postconditioning 2 test day ($p > 0.05$). Time spent on the food-paired side and the wheel-paired side during preconditioning and postconditioning was assessed in the ABA and FR groups. For the food-paired side, a two-way ANOVA revealed no significant main effect of time spent (Pre-CPP vs Post-CPP) ($F(1,6) = 0.621$, $p = 0.461$), and no significant main effect of ABA vs FR ($F(1,6) = 0.156$, $p = 0.706$) or a ABA vs FR x time spent interaction ($F(1, 6) = 0.248$, $p = 0.637$). For the wheel-paired side, a two-way ANOVA revealed no significant main effect of time spent (Pre-CPP vs Post-CPP) ($F(1,6) = 0.581$, $p = 0.475$), and no significant main effect of ABA vs FR ($F(1,6) = 0.162$, $p = 0.701$) or a ABA vs FR × time spent interaction ($F(1, 6) = 0.234$, $p = 0.646$). These results

indicate that there is no preference for the food-associated side or the wheel-associated side in the ABA or FR control groups (Figure 6). Further, there was no significant difference in time spent on either the food-paired or wheel-paired side when comparing preconditioning to postconditioning (test 1) and postconditioning (test 1) to postconditioning (test 2) in either the ABA or FR groups ($p > 0.05$, data not shown).

129/SvEv mice

Survival curves showing removal of each group from the ABA paradigm, along with the average percent baseline body weight on the final postconditioning (test 2) day for both ABA and FR groups, can be found in Figure 7. There was no significant difference between the ABA and FR groups in average percent baseline body weight on the postconditioning 2 test day ($p > 0.05$). Time spent on the food-paired side and the wheel-paired side during pre- and postconditioning was assessed in the 129/SvEv mice. For the food-paired side, a two-way ANOVA revealed no significant main effect of time spent (Pre-CPP vs Post-CPP) ($F(1,6) = 0.186$, $p = 0.681$), and no significant main effect of ABA vs. FR ($F(1,6) = 0.000$, $p = 0.976$) or ABA vs. FR × time spent ($F(1, 6) = 0.026$, $p = 0.877$). For the wheel-paired side, a two-way ANOVA revealed no significant main effect of time spent (Pre-CPP vs Post-CPP) ($F(1,6) = 0.205$, $p = 0.667$), and no significant main effect of ABA vs. FR ($F(1,6) = 0.000$, $p = 0.978$) or ABA vs. FR × time spent ($F(1, 6) = 0.024$, $p = 0.883$). These results suggest that ABA did not increase preference for the food-associated side or the wheel-associated side in the ABA or FR control groups (Figure 8). Further, there was no significant difference in time spent on either the food-paired or wheel-paired side when comparing preconditioning to postconditioning (test 1)

and postconditioning (test 1) to postconditioning (test 2) in either the ABA or FR groups ($p > 0.05$, data not shown).

General Discussion

Despite the known association between eating disorders and substance abuse disorders, it is not known if experiencing excessive weight loss during adolescence leads to neurobiological changes that later contribute to the development of substance abuse (O'Brien et al., 2003). The aim of this study was to investigate how activity-based anorexia affects the rewarding properties of methamphetamine and wheel running in two strains of female mice. Our results revealed that methamphetamine induced conditioned place preference in female C57Bl/6 mice but not female 129/SvEv mice, indicating that sensitivity to methamphetamine is strain-dependent. Furthermore, methamphetamine induced conditioned place preference in female C57Bl/6 mice that underwent the ABA model of anorexia nervosa, indicating that ABA enhances the rewarding effects of methamphetamine. Finally, in the modified condition place preference experiment, there was no preference for the food-associated side or the wheel-associated side in either the ABA or food restricted groups. Further, there was no significant increase in time spent on the food-paired side with animals in the food restricted group, indicating that perhaps the current parameters of the CPP model were not sensitive enough to detect a preference for either side.

Several theories have been proposed to explain the paradoxical hyperactivity that results from food restriction. During ABA, animals exhibit a significant drop in body temperature, a symptom also observed in patients with anorexia nervosa. It has been suggested that hyperactivity develops to counteract this drop in body temperature (Lambert, 1993). ABA has also been suggested to be the result of auto-addiction to endogenous opioids that are released during hyperactivity, and that dysregulation of the opioid system renders hyperactivity and self-

starvation behaviors as addictive (Marrazzi & Luby, 1986). Another intriguing explanation of ABA behavior comes from the foraging hypothesis, which is the idea that many species display increased locomotor activity and travel large distances during periods of food scarcity to find new food sources (Guisinger, 2003). If foraging behavior is rewarding in itself, then the chance that hyperactivity behavior will occur increases. As a result, the chance of survival of that species increases as well. This may be a biological explanation as to why hyperactivity may develop in mice that undergo the ABA model. Each hypothesis may contribute to the phenomenon and work in concert in the development of ABA.

To confirm whether the ABA model affected the reward system at all, we sought to explore whether the ABA model would increase the likelihood of methamphetamine-induced CCP in female C57/Bl6 mice in our second experiment. There is a strong association between the development of substance abuse disorders and the development of an eating disorder (Bahji et al., 2019; Brewerton et al, 2016; O'Brien et al, 2003). Psychoactive drugs and alcohol are often reported as being used by individuals with anorexia for their mood-altering effects; to escape, avoid, or numb; or to manage negative emotional states (Touyz et al.,2016). In animal models, food restriction often leads to increased self-administration of psychoactive drugs due to increased drug reward sensitivity and reward-related learning (Carr, 2011; Specker et al., 1994). Our findings indicated that rodents that undergo the ABA model may be more susceptible to the rewarding properties of methamphetamine and could be a steppingstone toward understanding the biological link between anorexia nervosa and substance abuse.

The results from Experiment 3 indicate that the hyperactivity seen in the ABA model may not reflect an increase in the rewarding effects of wheel running. Several studies have

implicated the involvement of the reward pathways within the ABA model (Avena & Bocarsly, 2012; Foldi et al., 2017a; Verty et al., 2011). Additional research has found an association between dopaminergic pathways and hyperactivity whereby antagonism of dopamine (DA) neurons leads to a reduction of hyperactivity (Klenotich et al., 2015; Verhagen et al., 2009a). However, other studies have demonstrated that food intake during ABA is associated with increased DA signaling and no similar increase is seen for periods of hyperactivity (Verhagen et al., 2009b). In the context of altered reward pathways, dopaminergic neuronal activation increases dopamine availability in the NAc to promote food intake and has no effect on hyperactivity (Foldi et al., 2017a). Our results from Experiment 3 seem to confirm that the hyperactivity behavior in the ABA model may not be perceived as rewarding. One limitation of Experiment 3 was its sample size; the null results of the experiment may be due to the experiment being underpowered. Additionally, the preference for the dark side at baseline that was observed in both groups may have limited the ability to detect an increase in preference when the wheel was placed on that side.

Given that the two strains tested in our study are commonly used background strains for transgenic mice, our findings provide insight into which strain might be most appropriate for future work testing the role of specific proteins in female mice that undergo the ABA model. For example, if the goal of a study is to test the hypothesis that removal of a specific protein prevents an enhanced methamphetamine-induced conditioned place preference with ABA, then females should be tested if the knockout mouse is of a C57Bl/6 background. Alternatively, if the hypothesis is that upregulation of a protein will enhance methamphetamine-induced conditioned place preference in ABA, then it might be easiest to detect this enhancement if overexpression

occurs in animals that underwent the ABA model and did not exhibit methamphetamine-induced CPP (i.e. females on 129/SvEv background).

Our work has laid the groundwork for future studies using conditioned place preference to test how ABA affects reward circuits. This line of research may provide important insights into the relationship between anorexia nervosa and addiction-like behaviors, a relationship that is often discussed in review articles but rarely tested directly. It is hoped that this work provides important clues regarding the neural basis of anorexia nervosa that will inform the development of novel treatments.

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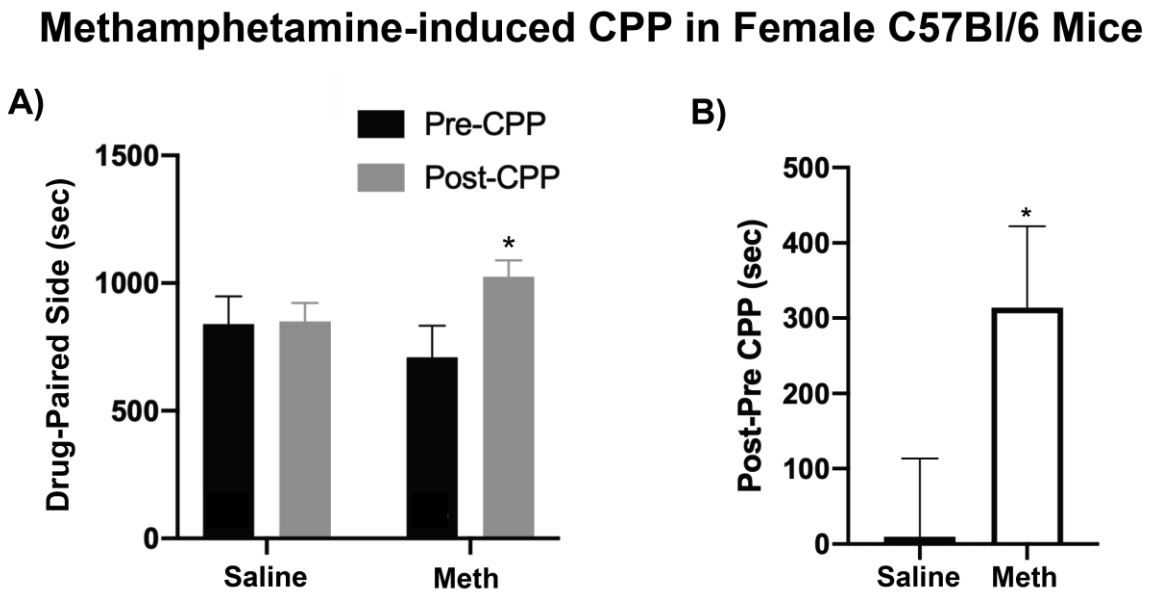

Figure 1. A) Mean time (seconds ± SEM) that female C57Bl/6 (n= 32) mice spent in the drug-paired compartment during preconditioning and postconditioning; *p < 0.05 versus preconditioning. B) Mean difference in time (seconds ± SEM) that female C57Bl/6 (n= 32) mice spent in the drug-paired compartment during preconditioning versus postconditioning; *p < 0.05.

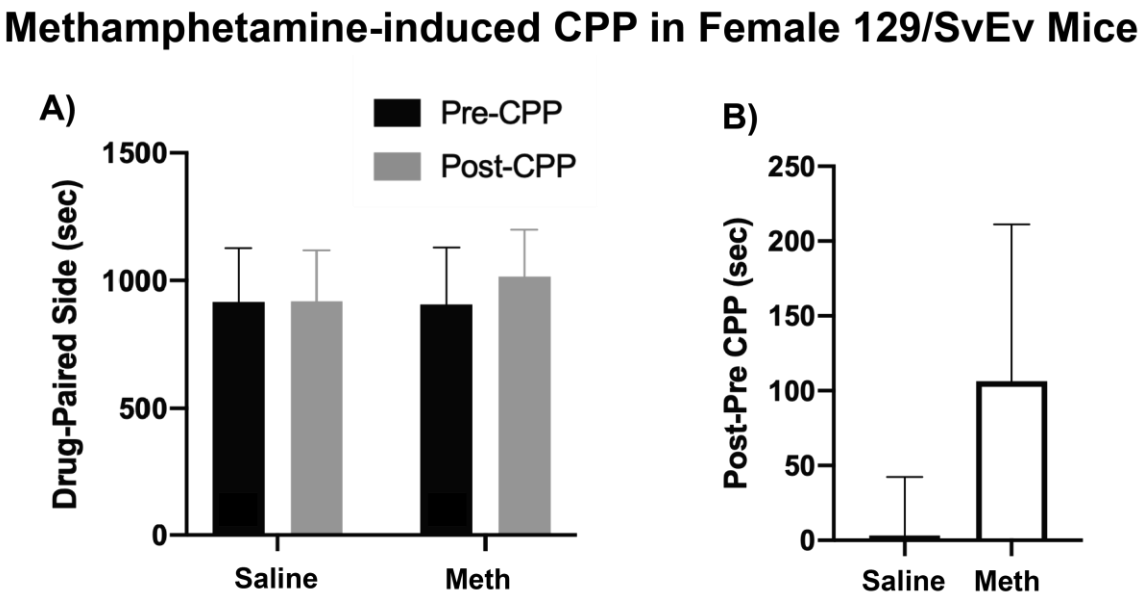

Figure 2. Mean time (seconds ± SEM) that female 129/SvEv (n= 32) mice spent in the drug-paired compartment during preconditioning and postconditioning; *p < 0.05 versus saline. B) Mean difference in time (seconds ± SEM) that female 129/SvEv (n= 32) mice spent in the drug-paired compartment during preconditioning versus postconditioning; *p < 0.05.

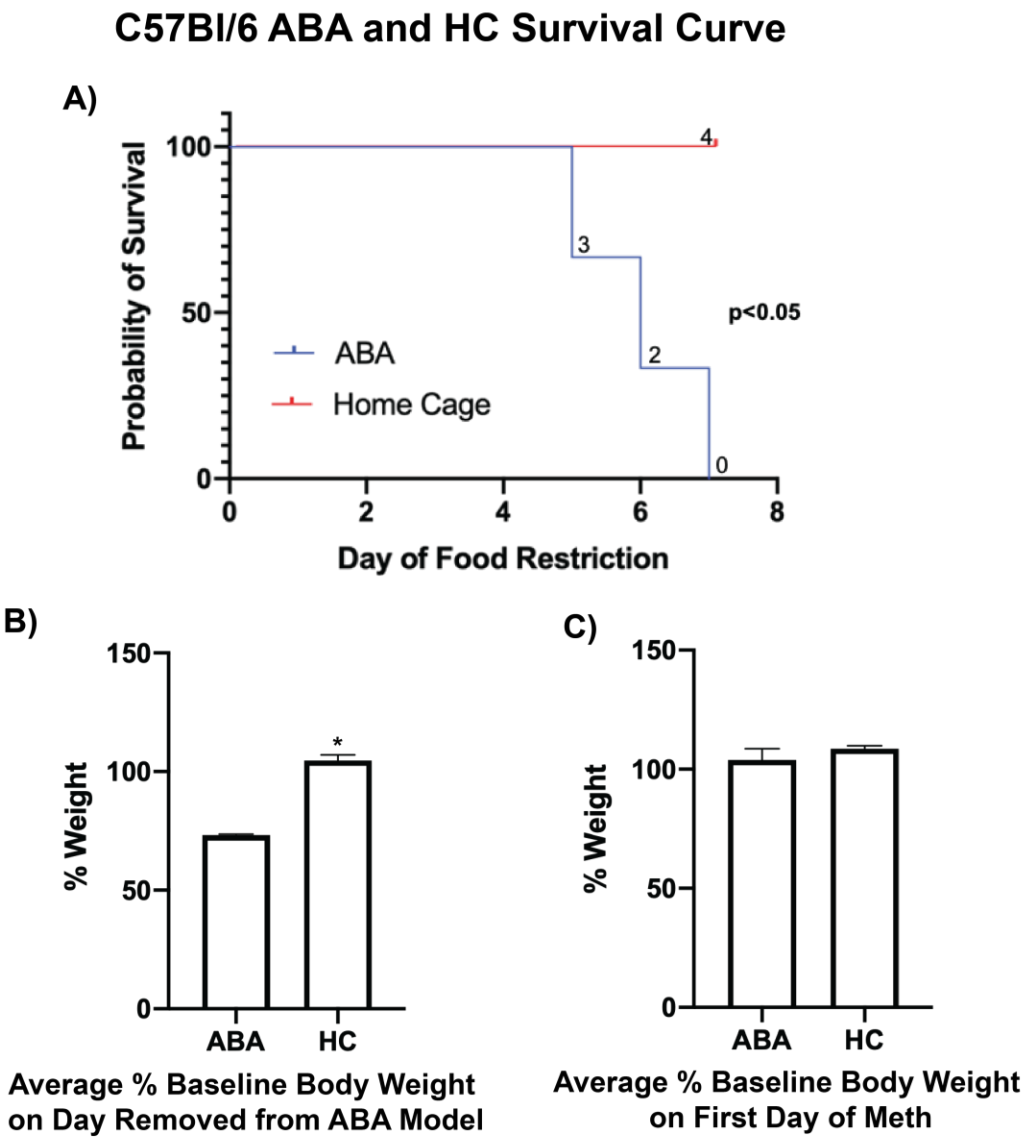

Figure 3. A) Probability of survival for female C57Bl/6 (n= 8) mice in the ABA versus HC group. B) Average % Baseline Body Weight (mean ± SEM) for female C57Bl/6 (n= 8) mice in Experiment 2. The weight of each animal on the day of ABA removal was divided by their weight on Baseline Day 4 of the ABA phase to find the average baseline % weight. C) Average % Baseline Body Weight (mean ± SEM) for female C57Bl/6 (n= 8) mice in Experiment 2. The weight of each animal on the first day of methamphetamine was divided by their weight on Baseline Day 4 of the ABA phase to find the average baseline % weight.

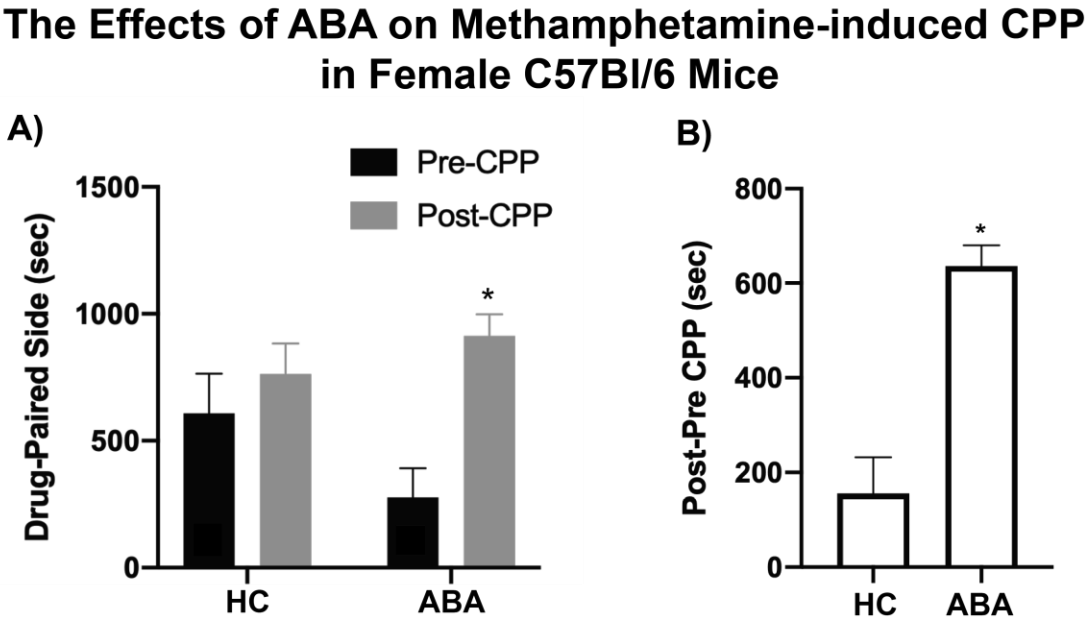

Figure 4. A) Mean time (seconds ± SEM) that female C57Bl/6 (n= 7) mice spent in the drug-paired compartment during preconditioning and postconditioning; *p < 0.05. B) Mean difference in time (seconds ± SEM) that female C57Bl/6 (n= 7) mice spent in the drug-paired compartment during preconditioning versus postconditioning; *p < 0.05.

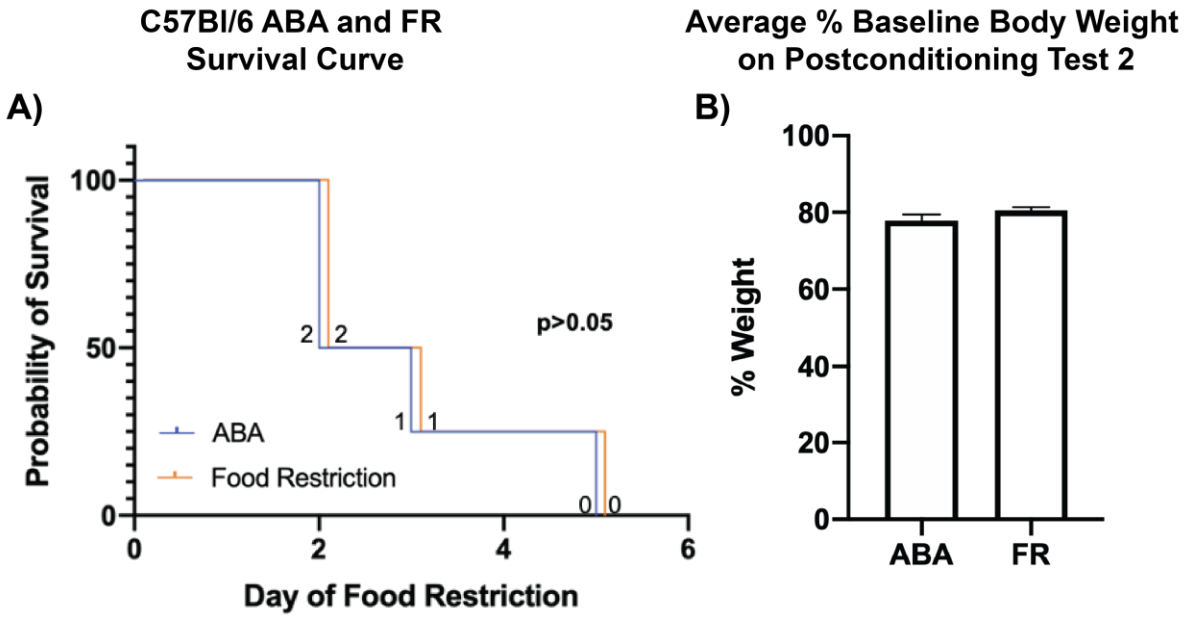

Figure 5 – A) Probability of survival for female C57Bl/6 (n= 8) mice in the ABA versus FR group. B) Average % Baseline Body Weight (mean ± SEM) for female C57Bl/6 (n= 8) mice in Experiment 3. The weight of each animal on the final postconditioning (test 2) was divided by their weight on Baseline Day 3 of the ABA phase to find the average baseline % weight.

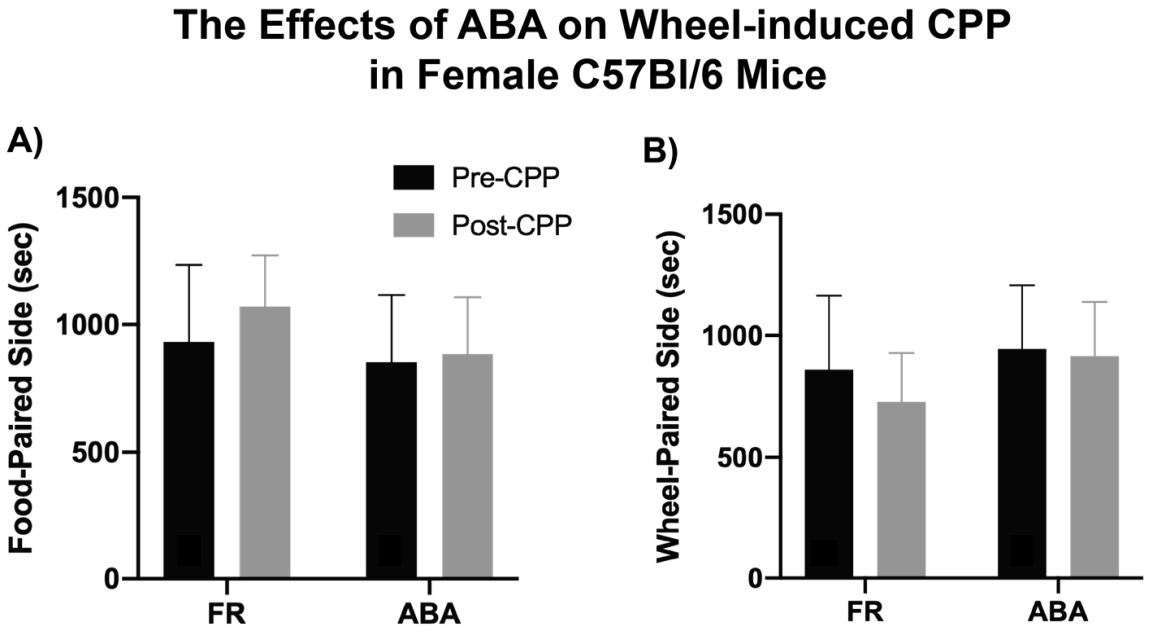

Figure 6 – A) Mean time (seconds ± SEM) that female C57Bl/6 (n= 8) mice spent in the food-paired compartment during preconditioning and postconditioning; *p < 0.05 versus FR. B) Mean time (seconds ± SEM) that female C57Bl/6 (n= 8) mice spent in the wheel-paired compartment during preconditioning and postconditioning; *p < 0.05 versus FR.

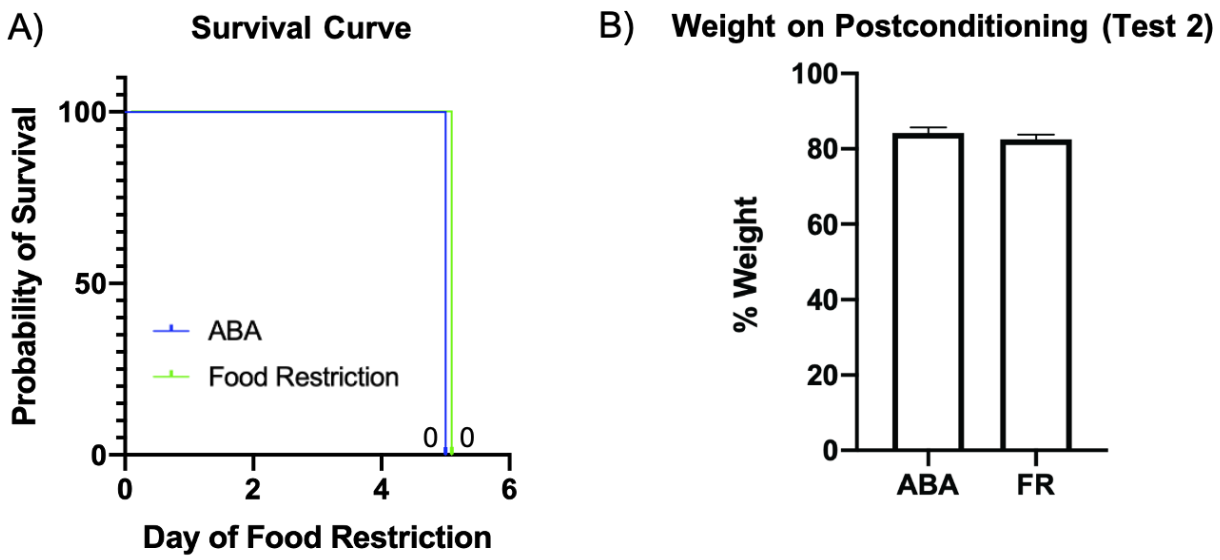

Figure 7 – A) Probability of survival for female 129/SvEv (n= 8) mice in the ABA versus FR group. B) Average % Baseline Body Weight (mean ± SEM) for female 129/SvEv (n= 8) mice in Experiment 3. The weight of each animal on the final postconditioning (test 2) was divided by their weight on Baseline Day 3 of the ABA phase to find the average baseline % weight.

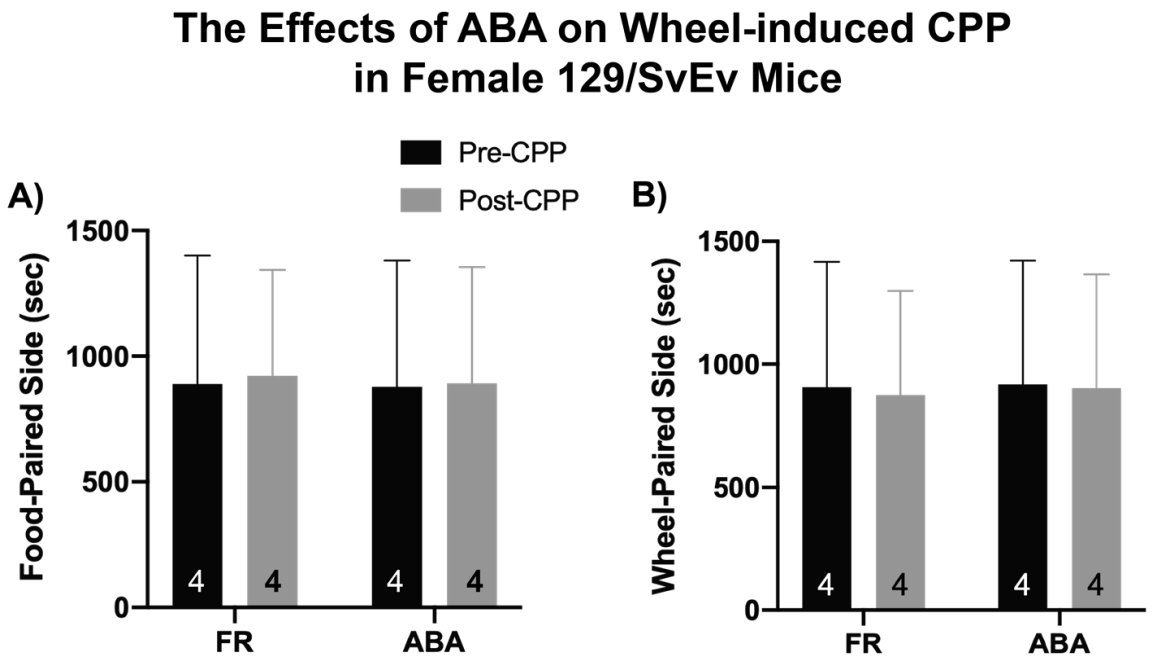

Figure 8 - A) Mean time (seconds ± SEM) that female 129/SvEv (n= 8) mice spent in the food-paired compartment during preconditioning and postconditioning; *p < 0.05 versus FR. B) Mean time (seconds ± SEM) that female 129/SvEv (n= 8) mice spent in the wheel-paired compartment during preconditioning and postconditioning; *p < 0.05 versus FR.