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An Exploration of the Wheel-Induced Feeding-Suppression

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by

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Abstract

Anorexia nervosa is an enigmatic human condition typified by food-restriction that is often accompanied by extensive exercise. This has been modeled in rats in the wheel-induced feeding-suppression (WIFS) model. In this model, animals are given access to a running-wheel, which induces a volitional drop in food-consumption. Short periods of wheel access have induced a feeding-suppression which is effectively reversed by chlorpromazine administration (Adams et al., 2009). Recent attempts at replicating Adams et al.'s (2009) feeding-suppression have, however, been unsuccessful (Peckham et al., 2013). These attempts raised questions as to whether or not the existing methodology is most effective at suppressing food-consumption in rats. A reliable WIFS model using short terms of wheel-access is important if drugs are to be tested in this paradigm. The first part of this thesis focused on which factors are most important for a WIFS to be seen and to use these findings to develop a model that can easily incorporate drug administration.

Experiment 1 tested if rats' body weights or their amount of running could predict the size of the WIFS. Experiment 1 explored the changes in food-consumption of 64 rats by providing 4 days of 24 h wheel-access followed by 4 days of 3 h wheel-access several days later. Neither body weight nor wheel-turns were predictive of the WIFS following 24 h or 3 h wheel-access. Experiment 2 sought to explore the effects of prior wheel-exposure duration on future wheel experiences. This experiment was a partial replication of Experiment 1; but with half of the rats (n =17) receiving 3 h wheel-access before 24 h wheel-access. It was found that the feeding-suppression was not evident in wheel naïve rats on the first day they received 3 h of wheel-access but was evident with 3 h access in rats with prior 24 h wheel experience. It was also found that the eventual feeding-suppression was larger with 24 h than 3 h of wheel-access.

Experiment 3 tested whether or not the time of day (morning or afternoon) that wheel-access is given was important to the WIFS which occurs over the subsequent 24 h and largely at night. This experiment provided 34 rats with 3 h wheel-access every third day for 4 wheel exposures. Time of wheel-access was found to affect running but not the feeding-suppression which was evident on each of the days following wheel-access. Experiments 1 to 3 led to the development of a paradigm used in Part 2.

Part 2 of this thesis explored the endocannabinoid system's (ECS) effects on the WIFS. Anorexia-like behaviours have been shown to directly affect the ECS. These changes in the ECS have been suggested as a sign of an underactive ECS in both humans (Gérard et al., 2011) and rats (Casteels et al., 2014). Interestingly, when cannabinoids are introduced to animals with wheel-access, food-consumption becomes elevated. This has been seen in a study using Δ9-tetrahydrocannabinol (THC), the main psychoactive ingredient in marijuana, where rats' weight-loss was attenuated by the drug (Verty et al., 2011). It has also been suggested that URB597, a drug that increases levels of anandamide (an endogenous cannabinoid comparable to THC), similarly 'restored' food-consumption (Peckham et al., 2013). Two experiments were conducted to independently examine these drugs in a new WIFS model. Experiment 4a focused on URB597 (0, 0.17, 0.5, and 1.0 mg/kg) whereas Experiment 4b focused on Δ9-tetrahydrocannabinol (THC; 0, 0.125, and 0.25 mg/kg) – both administered immediately after locked or unlocked wheel-access. This new procedure was effective in reliably inducing a WIFS but neither drug was able to prevent the feeding-suppression: suggesting cannabinoids might not play an important role in the WIFS.

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Anorexia nervosa (AN), the ultimate concern of the present studies, is a deadly eating disorder that involves a disruption in energy intake such that it becomes disproportionately low compared to the amount of energy that the person expends (Attia & Walsh, 2009; Casper, 2006; Hudson, Hiripi, Pop, & Kessler, 2007). AN is characterized by a dangerous reduction in food-consumption that can lead to unhealthy weight loss. This starvation is often accompanied by other behaviours such as intense exercise and hyperactivity or, in more severe cases, use of laxatives or self-induced vomiting that result in additional weight loss. Despite a patient's already low weight, these behaviours persist and exacerbate health issues (Hudson et al., 2007). In 1 of every 10 diagnosed cases of AN, death is predicted to result within 10 years. This is especially troubling as, among Americans, AN was found to be rather prevalent (0.9% in females and 0.3% in males). The present studies are designed to reflect (using rats) the simultaneous high-exercise and food-restriction seen in AN patients.

Currently, there is little understanding of the etiology of AN or how it can be treated. It is commonly speculated that exercise or food-restrictive behaviours may be causes or may be symptoms that need to be treated. As such, an animal model would be helpful to explore how food-consumption and exercise may interact or become a risk factor in the development AN. An animal model would allow early clinical tests of which neurological systems are involved in the increased activity and decreased food-consumption and if they are linked. An understanding of neurological systems would allow the testing of drugs in attempts to restore food-consumption to levels that are considered 'normal' and thus compensate for energy expenditure.

This thesis explores the possible role of endocannabinoids in eating disorders such as AN. Endocannabinoids are known to be involved in systems governing appetite and thereby food-consumption (i.e. Kirkham & Williams, 2001). A role of endocannabinoids in the disorder

has been discussed at length in more recent reviews (i.e. Palmiero Monteleone & Maj, 2013). Of interest to present research is the use of cannabinoid administration in models intended to reproduce anorexic-like behaviour in rats. Attempts to replicate previously successful paradigms, however, have discovered that methodology is yet to be fine-tuned. This thesis is thus split into two parts: Part 1 focuses on exploring factors important to the animal model; and Part 2 focuses on the injection of endocannabinoids into rats exposed to the procedure developed out of Part 1's findings.

Animal Models of Anorexia Nervosa

One rat model that seems to replicate the high levels of exercise and decreased food-consumption in AN is the activity based anorexia (ABA) model (Routtenberg & Kuznesof, 1967). This procedure typically provides rats with 23 h of access to a running-wheel followed by 1 h of access to food. In this paradigm, rats will continually increase exercise (quantified by wheel-turns) and decrease feeding (quantified by the amount of chow eaten) compared to rats receiving only wheel-access or restricted food-access, respectively. As a result of this increased exercise and decreased feeding, most rats will continually lose weight until death occurs (Routtenberg & Kuznesof, 1967). This model may be comparable to human AN since it reflects the discrepancy in energy balance (i.e. of energy intake and output) in rats. Rats in this model, similar to AN patients, 'voluntarily' decrease food intake while increasing exercise levels. Furthermore, rats can die in this paradigm much like in the human condition. Even though rats eat less than similarly food-restricted controls in this model, the use of food-restriction complicates the analysis of data as it is unclear if the effect is due to only 1 h food-access. Maybe 23 h of wheel-access only decreases food-consumption with 1 h food-access. Another issue with this model is that rats need to learn that food is only available for a short period in addition to

adjusting to wheel-access and therefore may not have time to adjust food-consumption to appropriately match energy expenditure.

An alternate model is the wheel-induced feeding-suppression (WIFS) model. In the WIFS model, rats are given up to 24 hours (Lattanzio & Eikelboom, 2003) of access to a running-wheel and ad lib food. Over the first few days of wheel-access, there is a dramatic drop in feeding which recovers over the following 8 to 10 days (Afonso & Eikelboom, 2003; Lattanzio & Eikelboom, 2003). The drop in feeding (typically 17-33%) is seen as a drop in comparison to baseline food-consumption or to a control group that is given continuous access to a locked-wheel. The advantage of a locked-wheel group is the ability to control for the effect of environment changes on food-consumption – we can determine that the effect is produced by running alone. The WIFS effect is counterintuitive as there is an imbalance between how much energy the rat is expending and how much the rat is eating. Rats that have the running-wheel are exercising and maintaining lower food-consumption than rats that are not exercising and continue to eat at baseline levels (Lattanzio & Eikelboom, 2003). Unlike the ABA model, rats are not at risk of dying in the WIFS model. Furthermore, decreased food-consumption in the ABA may be linked to a possible conditioned taste avoidance as wheel-access is paired with a small period of wheel-access. Decreased feeding seen in the WIFS has been shown to not be a form of conditioned taste avoidance (Satvat & Eikelboom, 2006).

While most work with the WIFS has used 24 h of wheel-access, a similar decrease in food-consumption is seen if short-term wheel-access is provided to rats. Lattanzio and Eikelboom (2003) showed that 2 h of wheel-access produces a WIFS nearly identical to that seen with 24 h of wheel-access. Since short-term wheel-access still causes a WIFS, this suggests that the decrease in food-consumption seen may not be entirely dependent on the absolute amount of

running as it is much lower with 2 h wheel-access (Lattanzio & Eikelboom, 2003). In fact, Afonso and Eikelboom (2003) found a non-significant relationship between the number of wheel-turns and the size of the feeding-suppression. These effects on feeding due to short, day-time wheel-access shows that rats are not simply running through times in which they would typically be consuming food (i.e. during the dark cycle). It is possible that a neural mechanism that reduces food-consumption is acted upon or instigated by the running behaviour.

Adams, Parfeniuk, & Eikelboom (2009) specifically tried to alter the WIFS by administering chlorpromazine (CPZ), an early antipsychotic, to rats using a short-access paradigm. CPZ affects multiple neurotransmitter systems (and potentially those that govern the WIFS). CPZ had previously been used in studies using the ABA model and had been shown to increase food-consumption but also decrease the running activity of rats (Routtenberg & Kuznesof, 1967). This allows the argument to be made that the decreased running may lead to a smaller decline in food-consumption. Therefore Adams et al. (2009) decided to use a WIFS paradigm that included only 3 h of day time wheel-access. This schedule of access allowed for a certain amount of experimental control. First, the locked-wheel groups allowed the researchers to target CPZ's direct effect on food-consumption. Secondly, administrations before wheel-access could provide information about how CPZ affects running. By controlling for the drug's effect on food-consumption and wheel-access independently, the researchers were able to target the WIFS – the interaction between wheel-access and food-access – with the drug. The researchers showed that in this paradigm the drug did not directly cause the attenuation of wheel-running or increase feeding but in animals with 3h wheel-access the drug itself prevented the WIFS. The prevention of the WIFS appears to be due to the dose used in this study (i.e. CPZ typically

affects wheel-running). The current thesis aims to replicate this achievement and broaden the types of drugs tested.

Endocannabinoids and the Wheel-Induced Feeding-Suppression

Recent research suggests that the endocannabinoid system (ECS) may be a involved in the reduced feeding effects seen in these two rat models of anorexia. The ECS is a retrograde signaling system that modulates synaptic communications (reviewed by Engeli, 2012) which have been found in both the central and peripheral nervous system. There is a larger concentration of CB1 receptors in the central nervous system and a larger CB2 concentration in the peripheral nervous system. CB1 receptors have been found to play a larger role in the modulation of food-consumption than CB2 receptors and are therefore a better target for drugs that may be used in the treatment of AN. For instance, CB1 agonists increase food-consumption whereas CB1 antagonists/inverse agonists decrease food-consumption. Most notable is SR141716 (or rimonabant): a CB1 receptor inverse agonist that was developed as an obesity treatment. It can effectively reduce food-consumption and appetite in humans and rats, but it has been shown to have depressive side-effects. Likewise, CB1 agonists have been shown to increase food-consumption. Therefore, it may be possible that a CB1 agonist could specifically prevent or reverse a change in food-consumption as seen in rats in the WIFS paradigm.

Work with humans suggests that the ECS has a role in AN (Frieling et al., 2009; Schroeder et al., 2012). Increased levels of CB1R (cannabinoid type-1 receptors) mRNA in AN patients suggests that AN creates a disturbance in the ECS. A role of the ECS is further supported by a reportedly higher occurrence of single nucleotide polymorphisms in genes coding for CB1Rs (Monteleone et al., 2009). This suggests that there may be a genetic predisposition to developing AN. Strong evidence of an altered ECS in AN patients comes from a positron

emission tomography (PET) study (Gérard, Pieters, Goffin, Bormans, & Van Laere, 2011). CB1Rs were marked in the human brain using a fluorescent CB1R ligand ([^{18}F]MK-9470), and AN patients were then scanned using PET. Underweight AN patients showed increased levels of CB1R availability in cortical and subcortical structures compared to healthy, non-AN controls (Gérard et al., 2011).

These PET findings in humans are directly reflected in research in which rats were subjected to the ABA paradigm (23 h wheel-access; 1 h food-restriction) and later injected with [^{18}F]MK-9470 and viewed via small animal PET techniques (Casteels et al., 2014). Rats in the ABA paradigm showed increased CB1R availability in both cortical and subcortical regions. When rats were taken out of the ABA paradigm and allowed to recover body weight and feeding, CB1R fluorescence returned to control levels. Rats that had been given access to a running-wheel for 10 days did not show increased CB1R availability. It is likely that testing 10 days after introducing wheel-access – at a time when rats would have mostly recovered feeding – allowed CB1R availability to return to normal. It is possible that if they had tested shortly after wheel-introduction the same CB1R upregulation might have been evident. Overall, the fact that results in human AN patients are mirrored in rats in an ABA model provides evidence that the ECS may in fact play a vital role in AN-related behaviour. This notion, however, is complicated by the fact that alteration of ECS signaling returns to 'normal' levels if weight and food-consumption are similarly restored. This indicates a possibility that these alterations may not be a cause of AN but an effect of AN – or starvation. If endocannabinoids, however, are capable of restoring food-consumption then it is nonetheless a possible treatment for patients with AN.

Verty et al. (2011) tested the ability of Δ9-tetrahydrocannabinol (THC; the primary psychoactive ingredient in marijuana) to reduce weight-loss in rats exposed to a variation of the

ABA paradigm (22.5 h wheel-access; 1.5 h food-restriction). They found that an injection of THC 30 minutes prior to the dark-cycle/food-access was able to increase weight relative to rats simply given vehicle. This study suggests a role of endocannabinoids in the ABA paradigm (Verty et al., 2011). An issue with this study is that there is no appropriate locked wheel control that received solely THC or vehicle outside of the ABA paradigm. It is possible that they are simply demonstrating THC's documented ability to increase food-consumption (Verty, McGregor, & Mallet, 2005) and not its ability to operate in behaviour modeling AN. It still, however, suggests positive evidence for a role of the ECS in eating disorders.

URB597, a drug that elevates endogenous cannabinoid levels, might be a drug to be considered as a potential treatment for AN symptoms. URB597 is a fatty acid amide hydrolase (FAAH) inhibitor. FAAH is an enzyme that rapidly metabolizes anandamide (AEA), an endogenous cannabinoid that has similarities to THC in receptor affinity (Grey, Terry, & Higgs, 2012) and effect (Solinas et al., 2007). Presumably URB597 administration can increase levels of AEA and other fatty acids involved in the regulation of food-consumption (Piomelli et al., 2006). Specifically, the elevation of AEA may be sufficient to increase food-consumption (Kirkham & Williams, 2001). Given the potential of URB597 as a treatment, it was studied recently by Peckham, Eikelboom, and Mallet (2013) in a procedure very similar to that used previously (Adams et al., 2009). URB597 was injected after either 2 or 4 hours of wheel-access (locked or open) at two doses. Results suggest that the highest dose of URB597 prevented the WIFS effect over a 24 h period (i.e. rats with wheel-access and URB597 ate the same amounts of food as when they had no wheel-access and URB597). However, this study failed to replicate the substantial drop in feeding in control groups shown in the model used by Adams et al. (2009) and Lattanzio and Eikelboom (2003) with shorter periods of wheel-access. Since this study

virtually replicated the design used by Adams et al. (2009) – 2 and 4 h wheel-access were used instead of 3 h wheel-access – this suggested a need for methodological refinement. This study does, however, suggest that Verty et al. (2011)'s work may have demonstrated not only THC's ability to increase food-consumption, but a potential for endocannabinoids to prevent the WIFS effect. Further research with URB597 and other cannabinoids is needed. Given these methodological issues there is a need to understand what variables are involved in producing the WIFS so that future studies can use a WIFS paradigm that is robust and always effective.

The first goal of this thesis was to develop a WIFS paradigm that would result in a reliable drop in food-consumption with less than 24 h of wheel-access. Having a model that shows suppressed feeding with smaller periods of wheel-access is important so that a drug can be injected either before or after locked or open wheel-access that could be used to disentangle the drug's effect on food-consumption and the drug's effect on wheel-running. Experiment 1, Experiment 2, and Experiment 3 were focused on achieving a WIFS paradigm to be used for future drug studies. The first experiment sought to run a large number of rats through a single WIFS paradigm to determine any predictors of the feeding-suppression. It was expected that wheel-turns would not be markedly important in predicting the WIFS (i.e. Afonso & Eikelboom, 2003) but that body-weight may be predictive of the WIFS. The second experiment was to compare different durations of wheel-access to see if any differences arise as a result of the duration of wheel-access and to see what effects previous wheel-access has on future experiences with the running-wheel. It was expected that 24 h wheel-access would lead to a larger WIFS and that 24 h provided before 3 h wheel-access would influence the size of the WIFS seen following 3 h wheel-access. The third experiment looked at whether or not the WIFS could be repeatedly induced with a 2 day break in between periods of wheel-access and if the time of wheel

introduction is crucial to the WIFS. It was predicted that the WIFS would grow with more days of wheel-access (Lattanzio & Eikelboom, 2003). No specific predictions were made for the time of wheel-access due to the exploratory nature of this experiment. Collectively, the findings of these studies indicate that there are no obvious predictors of the WIFS, that 24 h wheel-access creates a larger WIFS than 3 h of wheel-access, and that when rats start with 3 h of wheel-access the WIFS gets larger over days. Experiment 4 leveraged all of these findings to create a 6 h wheel-access paradigm using THC and URB597 as potential drug treatments for the WIFS. THC or URB597 were injected following wheel-access and it was hypothesized that it would replicate the findings in the study using CPZ. Findings with THC and URB597 demonstrate that this particular WIFS paradigm induces a substantial drop in food-consumption; however, neither drug prevented the WIFS. Experiment 4 suggests that a 6 h WIFS model can be used in future studies.

PART 1: DEVELOPING A WIFS MODEL

Experiment 1

Studies using short periods of wheel-access (Adams et al., 2009; Lattanzio & Eikelboom, 2003; Peckham et al., 2013) are inconsistent in their ability to induce a sizeable WIFS (Peckham et al., 2013). There are apparently aspects of the WIFS that are not well understood. Being able to predict the size of the WIFS can help wheel-running studies in terms of group selection and broadly in understanding how and why the WIFS effect varies. In this study it was expected that four days of 24 h wheel-access would induce a sizeable WIFS and that four days of 3 h wheel-access during the day would induce a WIFS (Lattanzio & Eikelboom, 2003). It was furthermore predicted that with 3 h of wheel-access it might take more than one day of access to induce a WIFS as there was no feeding-suppression with one day of 3 h wheel-access in the pilot study by

Peckham et al. (2013). Lattanzio and Eikelboom (2003) also found with 2 h of wheel-access it took more days to reach maximum feeding-suppression and reach a level comparable to feeding drops seen with 24 h wheel-access. Another purpose of Experiment 1 was to explore possible predictors of the WIFS. Wheel-turns and body-weight were used as variables that may predict the WIFS. It was expected that wheel-turns would not be predictive of the WIFS (i.e Afonso & Eikelboom, 2003) but that body-weight may be. Largely, this study was to identify factors (i.e. duration of wheel-access, wheel-turns, and body-weight) that are important to the expression of a WIFS.

Methods

Subjects. Sixty-four male Sprague-Dawley rats (565-896 g) were used in this experiment. Rats had previously been part of an experiment examining the effects of various quinine concentrations on sucrose consumption. Rats were individually-housed in a room with a 12 h light-dark cycle (lights on at 10:00 h) and had *ad libitum* access to food (Harlan Rodent Diet 8640) and water. The room had a temperature of 22±3˚C. This experiment and all following experiments in this thesis were approved by Wilfrid Laurier University's Animal Care Council which follows guidelines as laid out by the Canadian Council on Animal Care.

Apparatus. Wheel-cages (26.5 cm x 48.25 cm x 20 cm) were placed in the colony room of the rats. Nalgene running wheels (33 cm diameter x 11 cm width) were mounted in the cages and electronically connected to a computer running VitalView (version 4.0) by Minimitter.

Procedure. Measurements were taken of rats' food-consumption (over 24 h) and weights just after lights came on (10:00 h to 11:00 h each day). After a four day baseline (Days 1-4) rats were given continuous (24 h per day) access to a running-wheel for Days 5-8. Following this period of wheel-access, rats remained in their home cage for 6 days (Days 9-14). After this, rats

were given wheel-access for 3 h (11:45 h to 14:45 h) per day (Days 15-18). Baseline body weight and feeding measurements were continued for a two days after this final period of wheel-access concluded (Days 19 and 20). The data for the 64 rats was collected in two replications. Both replications (n=32) were run through the same procedure but the second replication was started 5 days after the first replication. As a result, the wheel-access for the second replication occurred when the rats in the first replication were not in wheels.

Data analysis. For each rat the four days of baseline feeding prior to each wheel-access period (24 or 3 h) were averaged. Differences between the average feeding for this baseline period and the feeding following wheel-access days were calculated and used as representations of the suppression and were used for calculating correlations involving possible predictors of WIFS (body weight and wheel-turns). Scatterplot/regression plots were created for the day of the most substantial drop in feeding and the associated weight and running. Two Day (first, to fourth of wheel-access) x Duration (24 h or 3 h) repeated-measures ANOVA were run using wheel-turns and feeding-suppression as independent variables. In both ANOVAs a replication factor was included to account for the fact that two replications were run. No significant effects or interactions of the replication factor were discovered so it is not discussed further in the results. All repeated measure statistics reported below and in following experiments are only reported significant ($p<.05$) if also significant after a Greenhouse-Geisser correction.

Results

One subject was not included in the analysis due to an error in the collection of the food data. Figure 1 shows 24 h food-consumption over the course of the experiment and Figure 2 shows the average number of wheel-turns on each day of wheel-access (for 24 h and 3 h). Means (±SEM) data used for analysis (baseline food-consumption, food-consumption following wheel-

access, the feeding-suppression, and wheel-turns) can be found in Table 1. The data from Day 6 and 17 were used for the 24 and 3 h correlational analysis.

The wheel-induced feeding-suppression. The repeated-measures ANOVA for wheel-turns showed significant effects of Duration, $F(1, 56)=294.89$, $p<.001$, Day, $F(3, 168)=102.25$, $p<.001$, and Duration x Day, $F(3, 168)=76.58$, $p<.001$. Figure 2 shows the main effect of Duration to be due to the rats running more with 24 h of wheel-access than with 3 h of wheel-access. It can also be seen, aside from the first day of 24 h wheel-access, that there is only a slight day-to-day change in running. That is, the first day of 24 h wheel-access saw more running than subsequent days of 24 h wheel-access – an explanation of the Duration x Day interaction.

The repeated-measures ANOVA of the feeding-suppression revealed a significant effect of Duration, $F(1, 61)=122.62$, $p<.001$, Day, $F(3, 183)=5.88$, $p=.001$, and a Duration x Day interaction, $F(3, 183)=6.51$, $p<.001$. Figure 3 would suggest that the significant effect of Duration is due to the 24 h of wheel-access inducing a larger WIFS than 3 h of wheel-access. The main effect of days, from Figure 3, suggests that the WIFS grows larger over days as rats eat less. Lastly, the interaction between Duration and Days suggests the possibility that, as is evident in Figure 3, it takes longer in the 3 h condition for a WIFS to become apparent. This is once again evidenced in Figure 1. Together, both ANOVAs (for wheel-turns and the WIFS) show that 3 h and 24 h access differ significantly. Furthermore, this difference in duration apparently affects how rats' feeding changes over days. The following results explore these differences in more detail.

24 h wheel-access. Rats weighed 567-908 g prior to wheel-access; a broad range of weights. Individual rats ran between 211-1175 wheel-turns with the first 24 h of wheel-access (Day 5). The rats feeding change after 24 h of wheel-access ranged from a decrease of 21.03 to

an increase of 5.08 g (mean change of -5.75 g). On the day of the largest WIFS (Day 6) rats ran between 64 and 851 wheel-turns. Rats showed a change in feeding ranging from -20.13 g to +0.2 g (mean -8.13 g). The number of wheel-turns on any given day of wheel-access did not predict the drop in food-consumption for that day (Figure 4 shows the relationship between wheel-turns and the feeding-suppression for Day 6). The weight of the rat prior to wheel-exposure was significantly correlated to the feeding-suppression on Day 5 of wheel-access (Figure 5). The lack of a significant correlation or correlations in the same direction on every day of the feeding-suppression, however, suggests that this is not a useful predictor. The correlation coefficients for these relationships can be found in Table 2.

3 h wheel-access. Rats weighed 584-924 g prior to 3 h wheel-access. For 3 h wheel-access, individual rats ran between 12 and 405 wheel-turns on Day 15 (the first day of 3 h wheel-access). The change in feeding ranged from -11.9 g to +10.95 g (mean of -2.09 g). On Day 17 (the day of the largest average feeding-suppression) rats ran between 22 and 343 wheel-turns. This day saw a change in food-consumption ranging between -13.33 g to +4.7 g (with a mean of -3.38 g). As with 24 h of wheel-access, the number of wheel-turns in 3 h was not predictive of the drop in food-consumption (see Figure 6). Rats' weight did not predict the drop in food-consumption from baseline in this 3h wheel exposure condition except on Day 18 (see Figure 7). As with findings with 24 h wheel-access, the lack of a consistent significant effect or consistent direction suggests once again body weight is not a useful predictor. The correlation coefficients for the 3 h wheel-access period can be found in Table 3.

24 h and 3 h wheel-access. As found in previous studies (Afonso & Eikelboom, 2003) running behaviour during 24 h periods of wheel-access was correlated with running behaviour during 3 h of wheel-access. Table 4 shows a matrix of this relationship. The level of feeding-

suppression induced by 24 h of wheel-access was not consistently related to the feeding-suppression following 3 h of wheel-access. The difference from baseline food-consumption after Day 6 and Day 8's wheel-access were significantly correlated to the suppression after Day 16's wheel-access (correlations, respectively are: r=.315, n=63, p=.012; and r=.437, n=63, p<.001). Suppression after Day 8's wheel-access was significantly correlated to suppression on Day 18's wheel-access: r=.345, n=62, p=.006). The fact that not all days of 24 h feeding-suppressions are related to 3 h feeding-suppressions shows that previous feeding-suppressions probably aren't able to predict future feeding-suppressions. Therefore, data from previous wheel experiences are not useful predictors of future data due to wheel-access. A full correlation matrix can be found in Table 5.

Discussion

To summarize the results: 24 h wheel-access produces a larger WIFS than 3 h wheel-access and no reliable predictors were found in this study. The number of wheel-turns on any given day of 3 h or 24 h wheel-access was not consistently correlated to the size of the feeding-suppression. The lack of a constant correlation between wheel-turns and the size of the feeding-suppression is consistent with previous work (Adams et al., 2009; Afonso & Eikelboom, 2003). This finding adds to an existing idea that the magnitude of running is largely unrelated to the size of the feeding-suppression. However, it is definitely shown in Experiment 1 that the duration of time rats have experienced the running-wheel influences the size of the feeding-suppression.

The weight of individual rats was not consistently related to the size of the feeding-suppression. Though some scattered correlations were found, the weight of the rat was not related to the size of the feeding-suppression on every day of wheel-access. The lack of a

relationship between rat weight and the size of the feeding-suppression shows that heavy rats are just as likely to show a sizeable WIFS as lighter rats.

The final set of correlations showed that the size of the feeding-suppression observed following 24 h wheel-access was also not consistently related to the size of the feeding-suppression observed following 3 h wheel-access. The lack of a maintained relationship between the feeding-suppression with 24 h wheel-access and 3 h wheel-access suggests that rats that show a large feeding-suppression after 24 h wheel-access are not the same rats showing the largest suppression following 3 h wheel-access. This finding is important in terms of group selection as it was thought this might a way to balance groups in a paradigm involving 24 h wheel-access pre-exposure followed by 3 h wheel-access (i.e. a procedure very similar to that used by Adams et al., 2009). This result shows that this is not a viable option.

The only reliable factor that was a strong predictor of the size of the feeding-suppression was the duration of wheel-access provided. It was shown that 24 h wheel-access showed a consistently larger feeding-suppression than 3 h wheel-access. For individual rats there is no maintained relationship between wheel-turns and the feeding-suppression. It is, however, unclear as to why a larger feeding-suppression is seen following 24 h wheel-access than 3 h wheel-access: the range in the running for these two durations of wheel-access overlaps. This suggests wheel duration may be more important than number of wheel-turns in understanding the WIFS. Rats may have a critical running duration after which they exhibit a robust drop in food-consumption seen with 24 h wheel-access. 24 h wheel-access then may be important as it allows rats enough time to run this duration.

There is, however, no suggestion that this is the case as previous research showed a nearly identical drop in food-consumption following either 2 or 24 h wheel-access (Lattanzio &

Eikelboom, 2003). A question that is left is whether or not previous experience with 24 h wheel-access affected rats' reaction to 3 h wheel-access. That is, rats would have been 'expecting' 24 h wheel-access when they were switched to 3 h wheel-access. A way to investigate this is tested in Experiment 2, where one group of rats were given 24 h wheel-access followed by 3 h wheel-access and another group of rats were given 3 h wheel-access followed by 24 h wheel-access.

Experiment 2

This experiment sought to examine how prior wheel-access affects the feeding-suppression induced by subsequent wheel exposure. In particular, does prior access to 3 h of wheel-access affect the degree of feeding-suppression induced by 24 h wheel-access and vice versa? It was predicted that 4 days of continuous 24 h of access to a running-wheel would reliably induce a large feeding-suppression from the first day of wheel-access whereas 3 h of daily wheel-access would require a few days before a marked feeding-suppression was observed. This prediction was based on previous work using short-term wheel-access (see Figure 1 from Experiment 1; see Lattanzio & Eikelboom, 2003). Experiment 2 examined 3 h and 24 h periods of wheel-access and their effects on food-consumption when provided to rats for 4 consecutive days. After two groups of rats had experienced either 3 h or 24 h wheel-access for 4 days, they were given a wheel-access duration they had not experienced before (i.e. 3 h wheel-access for rats that previously had 24 h wheel-access). The results show that 24 h of wheel-access induces a WIFS on the first day whereas 3 h wheel-access does not. When wheel-access was switched, both groups showed an immediate drop in food-consumption despite the duration of wheel-access. These results suggest that the novelty of the wheel must dissipate before a WIFS is observed.

Methods

Subjects. Thirty-four rats (511-725 g), previously used in an experiment focusing on sucrose consumption, were individually housed in a room with a 12 hour light-dark cycle (lights on at 10:00 h). Access to food (Harlan Rodent Diet 8640) and water were available *ad libitum* (i.e. food topped-up to at least 100 g every day) and the room was maintained at a temperature of 22±3˚C.

Apparatus. Same as in Experiment 1.

Procedure. Rats were divided into 2 groups with an equal number of rats from each condition in the previous sucrose experiment. Groups were defined by duration of initial wheel-access rats: the first group (n=17) received 4 days of 24 h wheel-access initially and then after a 6 day gap 4 days of 3 h access (Group L-S); and the second group (n=17) received 4 days of 3 h wheel-access initially and then after a 6 day gap of no wheel-access 4 days of 24 h wheel-access (Group S-L).

On days 0 to 3, baseline measurements of daily food-consumption and body weight were collected (all measurements occurred between 10:00 h and 11:30 h immediately after lights on for the rats). At 11:20-11:40 h on Day 4, after measurements were taken, rats were placed in wheels. If rats were part of the L-S group, they were given 24 h of wheel-access for the following four days (Days 4-7). If rats were part of the S-L group, they were given 3 h of wheel-access for the following four days (Days 4-7). On days of short-term wheel-access rats were removed from wheel cages at 14:20-14:40 h. All rats then had a 6 day break (Days 8-13). On Days 14-17 rats were once again placed in wheels. This time the L-S group received 3 h wheel-access whereas the S-L group received 24 h. Baseline measurements continued for two days after rats' final experience with the wheel. Rats were run in 2 replications that were 5 days apart. In

the end, there were 34 rats that had been given both 24 h and 3 h of wheel-access over four days: half receiving the longer duration first and half receiving the shorter duration first.

Data analysis. Using daily wheel-turn totals or feeding change from the baseline average as the independent variables, two Duration (24 h or 3 h wheel-access) x Day (Day 1 to 4) repeated measures ANOVA was run with Order (24 h or 3 h wheel-access first) as the between-subjects factor. Day 4 was excluded for analysis for wheel running since one group of the Order variable (17 rats) was missing wheel-running data for this day. Subsequently, one-way ANOVAs were carried out on all days of 3 h and 24 h wheel-running using Order (whether rats had 3 h of wheel-access first or 24 h of wheel-access first) as a factor.

Results

An error in the Vital View data collection software caused approximately 12 hours of wheel-data to be lost on the Day 17 of 24 h wheel-access (for one replication of the S-L group). This error resulted as the computer failed to collect wheel-turn data – however rats were still running. Baseline feeding and the change in feeding from the baseline average were calculated in an identical manner to Experiment 1. All daily averages for data used in the subsequent analyses can be found in Table 6. The wheel—turns for 24 h and 3 h wheel-access are shown in Figures 8 and 9, respectively. The WIFS for both 24 h and 3 h wheel-access can be found in Figures 10 and 11, respectively.

24 h and 3 h wheel-access. For the ANOVA using wheel-turns as an independent variable, a significant effect of Duration, $F(1, 16)=174.155$, $p<.001$, Day, $F(2, 64)=67.814$, $p<.001$, Duration x Order, $F(1, 32)=4.303$, $p=.046$, and Duration x Day, $F(2, 64)=56.93$, $p<.001$, were found. The main effect of Duration suggests, not surprisingly, that the amount of running is higher with 24 h than with 3 h of wheel-access (see Figure 8 and 9). The same figures suggest

the Day effect is due to a running decrease on subsequent days of wheel-access. Figures 8 and 9 suggest that the Duration x Order interaction may be due to the fact that running in the second wheel exposure is slightly higher than the average in the first exposure. This difference is mainly seen in a comparison of the two groups on the first day of 24 h wheel-access. Lastly, the Duration x Day appears to be due to rats decreasing their running over days with 24 h access (i.e. this trend is not seen with 3 h wheel-access). For one-way ANOVAs for each day of wheel-access, no significant differences were found between rats which had the 3 or 24 h first or second.

The repeated measures ANOVA on the feeding change from the baseline average showed only a significant effect of Duration, $F(1, 32)=35.53$, $p<.001$, and a Duration x Day x Order interaction, $F(3, 96)=3.829$, $p=.012$. From Figures 10 and 11 it is evident that 24 h of wheel-access produces a larger decrease in feeding from the baseline average than does 3 h of wheel-access. The three-way interaction (Duration x Day x Order) suggests that the decrease in food-consumption in the 3 h condition is not evident in the first day if this is the first exposure to the wheel but is evident if rats had prior wheel-access. With 24 h of wheel-access there is always a feeding-suppression. This suggestion is supported by the one-way ANOVAs comparing Order (whether rats had 3 h of wheel-access first or 24 h of wheel-access first) that were carried out on the feeding decreases for each day of 3 h and 24 h wheel-access. The only significant difference was on the first day WIFS of 3 h wheel-access, $F(1, 32)=11.11$, $p=.002$, only in wheel experienced rats does 3 h wheel-access cause a WIFS. This suggests that previous experience with a wheel may be necessary to induce a feeding-suppression with smaller durations of wheel-access.

Discussion

In terms of wheel-running, longer access resulted in more running and the amount decreased over days. The fact that longer wheel-access leads to more running is both intuitive and cohesive with previous research findings (i.e. Lattanzio & Eikelboom, 2003). The decrease in running over days is different from what is seen with younger rats where running increases over days (Lattanzio & Eikelboom, 2003). In this light, it is interesting that the feeding-suppression remains stable over days. This is another indication that the absolute amount of running is not critical to induce the feeding-suppression. The fact that 3 h wheel-turns remain relatively consistent compared to 24 h wheel-access is also cohesive with previous findings (Lattanzio & Eikelboom, 2003).

Of particular interest in this study are the findings based on the change from baseline food-consumption after wheel-access. It was found that 24 h wheel-access produced a larger decrease in food-consumption from baseline than 3 h wheel-access did. It was evident that 3 h wheel-access did not immediately induce a substantial decrease from baseline feeding unless rats had some prior experience with the running-wheel. The fact that 24 h produced a larger decrease from baseline food-consumption than 3 h wheel-access does not parallel what has previously been shown (Lattanzio & Eikelboom, 2003) but is consistent with what was found in Experiment 1. It is unclear whether this suggests that a larger WIFS can be induced if longer wheel-access is provided. It is also an interesting finding since it has been continually shown in a variety of ways that the number of wheel-turns is not related to the size of the WIFS. A possible explanation of this finding is twofold: one, that there is a critical duration of running that rats must reach before a WIFS is expressed; and two, that there is a learning component involved in the expression of a WIFS. In other words, the presence of the wheel must lose its novelty before a WIFS is seen.

This interpretation also applies to the complex relationship between the feeding-suppression, previous wheel-exposures, duration of the wheel-exposure, and the number of days of wheel-access. For example, a possible reason that a WIFS was not seen on the first day of 3 h wheel-access for the S-L group was that the wheel's novelty had not yet dissipated. That is, 24 h wheel-access is long enough for the wheel's novelty to dissipate whereas 3 h is not. If this is true, then this would also explain why the WIFS is not present in rats that have their first experience with 3 h wheel-access but with subsequent periods of 3 h wheel-access. This lack of novelty would explain why the rats the L-S group showed a feeding-suppression on the very first day of wheel-access: the wheel was no longer novel. Therefore this finding may simply be explained by the apparent fact that simply having previous experience with any form of wheel-access will lead to a feeding-suppression when rats are presented with 3 h wheel-access. Performing an experiment using 3 h of wheel-access followed by subsequent periods of wheel-access would allow for a further understanding how repeated periods of wheel-access affect the WIFS. It is also not understood why 24 h of wheel-access provides a larger WIFS than 3 h wheel-access. It may be possible that 24 h of wheel-access allows rats to run at a time they are most susceptible to the WIFS. Experiment 3 sought to explore this in more detail: providing rats with 3 h of wheel-access on days separated by a gap either early in the light-cycle or later in the light-cycle.

Experiment 3

Experiment 2 showed that 24 h a day continuous wheel-access was not necessary to see the WIFS. With only 3 h of wheel-access the first wheel exposure did not result in a WIFS but when rats had prior wheel experience the 3 h wheel-access induced a feeding-suppression – even when preceded by a gap of several days. It also showed that the feeding-suppression could be reinstated after a several day gap in wheel-access – whether or not 3 h or 24 h wheel-access had

come before. Experiment 3 explored if a WIFS is maintained if short wheel-access was interspaced with a couple days without wheel-access. It also explored if the size of the WIFS is affected by the time of day rats are given wheel-access. This experiment involved rats receiving wheel-access for a 3 h period once every three days – either in the 'morning' or in the 'evening'. The results suggest that more than one day of 3 h wheel-access is required before a feeding-suppression becomes robust.

Methods

Subjects. Thirty-four rats (420-679 g) that were previously used in an experiment focusing on sucrose consumption were single-housed in a room with a 12 h light-dark cycle (lights on at 10:00 h). Rats had *ad libitum* access to food (Harlan Rodent Diet 8640) and water. The room had a temperature of 22±3˚C.

Apparatus. Same as in Experiments 1.

Procedure. Rats were divided into two separate groups (based on previous research experience such that an even number of previous group assignments were in each group): Early and Late. The Early rats would receive wheel-access early in the light cycle (the 'morning') whereas the Late group would receive wheel-access later in the light cycle (the 'evening'). Each period of wheel-access lasted 3 hours. Early rats (n=17) were placed in wheels at 11:30 h (1.5 h after lights came on) and taken out of wheels at 14:30 h once very third day for 4 exposures. Rats were run in 3 replications so for each group 6 rats were placed in wheels the first day, 6 on the second day, and 5 on the third day. This procedure was repeated every third day until all rats had a total of 4 exposures to a running-wheel. This same method was used for the Late group; however, those rats were given 3 h wheel-access at 17:30 h and were taken out at 20:30 h (1.5 h before lights went off).

Data analysis. Food-consumption was calculated in a way identical to Experiment 1. Since wheel-access was provided every three days, a baseline average of the two days was used. A mixed measure Day (Day 1-4) x Time (Early or Late) ANOVA was carried out for both the feeding-suppression and wheel-turns. Since rats were run in three replications, a replication factor was initially included in all ANOVAs but was never significant.

Results

All data used for analyses, actual baseline feeding for each cycle, feeding on the day after wheel-access, the feeding-suppression and the number of wheel turns, can be found in Table 7. For the ANOVA on wheel-turns, there was a significant effect of Time, $F(1, 28)=15.33$, $p=.001$. Figure 12 shows the running in the Early and Late groups over each of their wheel experiences and it is evident that rats ran more in the wheels in the morning. The day-by-day food-consumption graph for the two groups can be found in Figure 13 and Figure 14 shows the feeding-suppression induced. The ANOVA for the feeding-suppression showed only a significant effect of Day with $F(3, 78)=5.066$, $p=.003$. From Figure 14 it is evident that the feeding-suppression becomes more evident across days of wheel-access but seems similar in the early and late group rats.

Discussion

Since rats do most of their food-consumption at night, the results of this experiment suggest that wheel-access early in the day or close to the time of peak feeding induce similar drops in food-consumption. Therefore, the time of wheel-introduction during the light-cycle does not influence the size of the feeding-suppression. Even though the time of day in which wheel-access was introduced played in important role in how much rats ran it did not play a role in how much rats decreased food-consumption following wheel-access (that is, both groups' drop in

food-consumption were nearly identical). This further supports the notion that wheel-turns are not related to the size of a feeding-suppression.

The results of this experiment also show that the more experience a rat has with a wheel, the more pronounced the WIFS becomes. Particularly, the third day of wheel-access appears to be the day with a pronounced feeding-suppression. This is consistent with previous work that also shows the third day as a 'peak' WIFS (Lattanzio & Eikelboom, 2003).

Another way to conceptualize the findings from this study is that it takes at least 6 h of wheel-access before a feeding-suppression will become evident. Therefore, it may be possible to hasten the feeding-suppression by giving rats more time in the running-wheel per day. The lack of sufficient prior running experience may be why Peckham, Eikelboom, and Mallet (2013) failed to find a robust WIFS in their paradigm. As such, any future studies looking to use the WIFS paradigm should seek to increase the time of wheel-access above 3 h with at least one day of wheel-access prior to studying the WIFS. This is precisely what was done in the following experiments.

PART 2: THE ROLE OF CANNABINOIDS IN THE WIFS

Given the findings of the three previous experiments, a WIFS paradigm was developed in which rats would receive access to a locked or unlocked running-wheel for 6 h once every three days. To begin to understand the pharmacology of the WIFS, two cannabinoids were tested. URB597 (a FAAH-inhibitor that indirectly increases levels of anandamide) had previously been suggested to influence the WIFS in a dose-dependent manner (Peckham et al., 2013). Similarly, chronic Δ9-tetrahydrocannabinol (THC; a psychoactive ingredient in marijuana) has been shown to attenuate weight loss precipitated by wheel-access (Verty et al., 2011). In order to clarify recent findings (i.e. Verty et al., 2011) concerning cannabinoids and wheel-running, it was

decided to test these two drugs in a WIFS paradigm. This experiment took into account past findings that no predictors of the WIFS were identified (Experiment 1), more than 3 h of wheel-access is required before the WIFS becomes notable (Experiment 2 and 3), and that the WIFS can be induced in an every third day paradigm (Experiment 3). To incorporate these findings, rats were given wheel-access (locked or unlocked) every third day for 6 h on each day after all rats received a 6 h pre-exposure day. This gap was provided so that any lingering effects of the drugs would dissipate before the next day of testing. Rats received doses of THC (0, 0.125, or 0.25 mg/kg) or URB597 (0, 0.17, 0.5, or 1.0 mg/kg) – in two separate experiments. These doses were selected as they were previously shown to effectively increase consumption of margarine (Scherma et al., 2013) and were injected immediately after wheel-access (2 h before onset of the dark cycle). This time was chosen as URB597 causes peak AEA levels after about 2 h (reviewed by Piomelli et al., 2006). THC, on the other hand, reaches peak levels after 30 minutes (reviewed by Verty et al., 2011). THC was injected at the same time as URB597 to control for the time of injection. It was predicted that wheel-access would decrease food-consumption on days when rats are injected with vehicle relative to days when rats are given the locked wheel and vehicle. It was also predicted that THC and/or URB597 would dose-dependently prevent the WIFS on days of wheel-access but not affect feeding on days of locked wheel-access.

Experiment 4a: URB597

Methods

Subjects. Fifteen male, Sprague-Dawley rats (200-225g) were individually housed in a room on a 12 h light-dark cycle (lights on a 9:00 h). Rats had access to food and water *ad libitum* in a room with a temperature of 21±2ºC.

Apparatus. Same as that described in Part 1.

Drugs. URB597 was suspended in a vehicle of PEG-400, TWEEN 80, and physiological saline (1:1:18). Initially, URB597 was placed in a small amount of saline and PEG-400 and stirred for several minutes. Following this more saline and TWEEN 80 were added and stirred together. Through this process a single, large dose of URB597 was created. This large dose was then diluted using the vehicle (PEG-400, TWEEN 80, and physiological saline) so that 3 active doses were generated: 0.17 (LOW), 0.5 (MID), and 1.0 mg/kg (HIGH). The vehicle (VEH) itself was also injected. All injections were made intraperitoneally (i.p.) in a volume of 1 ml/kg.

Procedure. Four days of body weight and food-consumption were collected. Measurements of weight and food were conducted at 10:00±00:15 h each day. All rats received a single pre-exposure to a running-wheel for 6 h (13:00±00:10 h to 19:00±00:10 h). Three days after this initial access, rats were randomly assigned to receive access to a locked or unlocked running-wheel and one of the doses of URB597. All URB597 injections occurred immediately after rats were taken out of the running wheels (19:00±00:10 h; i.e. 2 hours before lights-off). Every third day, rats received access to a locked or unlocked running-wheel and a single dose of URB597 – the combination of wheel (locked or unlocked) and dose would be one that the rat had not experienced prior. A Latin Square design was used to randomize the order of exposure to drug and wheel-access combinations. This procedure was carried out for a total of 8 days (4 URB597 doses x locked or unlocked wheel-access). At the study's conclusion every rat had received one day of pre-exposure, 4 days of locked wheel-access, 4 days of open wheel-access, and each dose of URB597 twice.

Data analysis. The feeding-suppressions were calculated in a manner identical to previous experiments – using only two days of baseline food-consumption. A repeated measures ANOVA was run on wheel-turns using Dose as the within-subjects factor. Another repeated

measures ANOVA using Wheel-access (locked or unlocked) x Dose (0, .17, .5, and 1.0 mg/kg) was run on the feeding-suppression on these days (24 h after wheel-access). Since two replications of animals were run, a factor coded for days of replication (first day or second day) was tested and was found to have no significant interactions or main effects and is thus not reported below.

Results

One rat was removed from analysis due to continually low feeding resulting from broken teeth thus all analyses were carried out using data from 14 rats. Table 8 shows the means (±SEM) of data used for analysis of these results of these 14 rats' 2 day baseline food-consumption, food-consumption following wheel-access, feeding-suppression, and wheel-turns.

An examination of the equipment used for running revealed that 1 wheel was counting singular wheel-turns as 2 wheel-turns. Though this was not happening for every single wheel-turn, it happened consistently enough for it to be able to affect the total number of wheel-turns counted for the rats. Ultimately this meant the 2 rats that used this piece of equipment had elevated levels of running. These levels would have affected the mean number of wheel-turns. Since this study used a within-subjects design this was not cause for concern. The consistent double-counting would have ensured that the measure was a reliable indicator of running activity. Furthermore, the focus of this study was not wheel-turns but the decrease in food-consumption. The accuracy of wheel-running data is, therefore, not necessary since the primary concern was simply whether or not rats had access to the running-wheel. It is also not a concern as any drug injection after wheel-access was, not surprisingly, found to have no effect on wheel-turns. This suggests that there was no difference between rats in how much they were running on any given day. The wheel turns for the days of wheel-access are shown in Figure 15. A repeated

measure ANOVA of all four drug doses revealed no significant differences across the doses. So, despite the technical error, which appeared to result in a constant elevation of the count, the data was found to be reliable.

Figures 16 and 17 show the average food-consumption and feeding-suppression, respectively, based on food-consumption following either locked or unlocked wheel-access and the dose of URB597 injected. A repeated-measures Dose x Wheel-access ANOVA conducted on feeding-suppression data showed only a significant effect of Wheel-access, $F(1, 12)=32.08$, $p<001$. This suggests that access to an unlocked running-wheel significantly reduced food-consumption. The lack of any other significant findings suggests that URB597 used at these doses that it has little effect on absolute feeding. These results furthermore suggest that these doses of URB597 administered in this procedure are not effective at preventing the WIFS.

Experiment 4b: The Role of THC in the WIFS

Methods

Subjects. Fifteen male, Sprague-Dawley were kept in conditions identical to Experiment 4a.

Apparatus. Same as Experiment 4 and all experiments described in Part 1.

Drugs. THC in absolute ethanol (50 mg/ml) had a small amount of a TWEEN 80 and ethanol mixture (1:1) added to it and was then stirred for several minutes under a nitrogen flow. After the last of the ethanol evaporated, physiological saline was added and stirred for several more minutes. The result was a dilution which had THC diluted in 1% TWEEN 80 and physiological saline. Dose of THC produced were 0 (VEH), 0.125 (LOW), and 0.25 mg/kg (HIGH) was administered i.p. to the rats.

Procedure. The procedure was identical to that in Experiment 4; however, instead of URB597, only 3 doses of THC were injected. As a result, this design involved 3 days of locked and 3 days of unlocked wheel-access.

Data Analysis. Same as in Experiment 4a. Once again, since two replications were run, a factor to account for this was tested and was found to not have any significant main effects or interactions.

Results

Table 9 shows the means (±SEM) of data used for analysis of these results of the 15 rats' 2 day baseline food-consumption, food-consumption following wheel-access, feeding-suppression, and wheel-turns.

Once again, running was found to be constant over days as no significant effects were found in the ANOVA conducted on wheel-turns. (i.e. no day-to-day difference). Figure 18 shows the means (±SEM) of wheel-turns on each day of wheel-access and supports this conclusion.

Mean food-consumption on days of locked and unlocked wheel-access can be found in Figure 19; the feeding-suppression itself can be seen in Figure 20. For the repeated-measures ANOVA on the feeding-suppression, there was only a significant effect of Wheel-access, $F(1, 13)=28.63$, $p<.001$. The significant effect of wheel-access shows that being able to run with wheel-access was responsible for the expression of a WIFS – suggesting a working model. No results suggest that THC directly impacted feeding, irrespective of wheel-access, as is clear in Figures 19 and 20. There is a small interaction effect suggested in Figure 20; however, this effect is non-significant, $F(2, 12)=.48$, $p=.63$. As found in Experiment 4a, no significant effects were found that would suggest THC made the WIFS larger or smaller.

Discussion of Experiment 4a and 4b

To summarize both of these studies: it was found first that the paradigm used was effective in producing a feeding-suppression. It was also found that when wheel-access is given in this paradigm that the amount of running is constant across all days of wheel-access. Lastly, neither of the drugs apparently had an effect on the decrease in feeding from baseline following wheel-access. That is, neither the 3 doses of URB597 nor the 2 doses of THC used in Experiments 4a and 4b had on effect on the size of the WIFS.

The finding of a paradigm capable of inducing a feeding-suppression is valuable as previous research had issues replicating past paradigms (Peckham et al., 2013). The present paradigm simply expanded on previous paradigms and used findings from Experiments 1, 2, and 3 to arrive at a paradigm that involved a 6 h pre-exposure followed by further 6 h wheel-access periods every third day. The suppression induced by 6 h wheel-access was able to be reinstated after a 3 day gap in wheel-access and was maintained over multiple drug doses. In other words, the effect was relatively stable. Another finding in this paradigm is that running was consistent over days of wheel-access. Since there is no escalation or decline in the number of wheel-turns in this paradigm, future studies looking at the effects of drugs on the WIFS can be assured that the effects they see are independent of wheel-turns. Therefore Experiment 4a and 4b have demonstrated a reliable WIFS model that should be used in future drug testing studies.

The other finding of this study showed that neither of the drugs used – or potentially the doses they were used at – were effective at altering the size of the feeding-suppression. In Experiment 4a URB597 was used. URB597 is a FAAH-inhibitor that increases the amount of the endogenous cannabinoid, anandamide, and other fatty acids. Conversely, in Experiment 4b THC was used. THC is an exogenous cannabinoid. In sum, Experiment 4a and 4b tested the effect of

both exogenous and endogenous cannabinoids on the WIFS. Neither drug appeared to have a direct effect on food-consumption on days where the wheel was locked (i.e. 'normal' feeding). On days of wheel-access drugs did not appear to have a direct effect on the WIFS (i.e. 'normal' feeding-suppression was seen). It is difficult to say conclusively whether or not either drug is completely ineffective in terms of altering the WIFS or if it was simply the doses selected in these studies that were ineffective. A way to answer this would be for future research to broaden the range of doses administered. A further way to answer this would be for research to broaden the types of drugs being administered: perhaps including other drugs from the family of early antipsychotics (Adams et al., 2009).

Another interesting idea that arises from these two studies is what role the time of administration plays in the WIFS. For instance, it is possible that the time of administration (immediately after wheel-access) was not effective in preventing the WIFS. It is unknown if the WIFS has a 'critical time' in which a drug must be administered in order to stop it. It is also unknown if the WIFS effect can be reversed once it has been instigated. This is a more complex question as it would require mapping out a temporal course of the WIFS and finding a time when the WIFS begins. A way to test this would be to take hourly food measurements and to make an attempt to reverse the WIFS after the first decrease from 'normal' feeding is seen. Conversely, if the time of decrease is known, a drug may be administered prior to this time point in an attempt to prevent the WIFS from occurring.

There are obviously many questions left open from this study. It can, however, be said that in this paradigm that URB597 and THC were ineffective at the doses used and/or the time administered. A broader range of drugs and/or doses could be used in future research to expand the current understanding of what neurological systems may be involved in the WIFS. The

paradigm itself was effective at inducing a lasting WIFS – which has been a primary aim throughout this thesis. Future research should attempt to further understand the WIFS using this paradigm that involves 6 h pre-exposure prior to subsequent testing day in which 6 h wheel-access is also given.

GENERAL DISCUSSION

There were two main conclusions of this series of experiments each with a number of practical and general implications. First was the development of a workable model of limited wheel-access which resulted in a reliable feeding-suppression. Second were the two initial tests of the hypothesis that the endocannabinoid system might play a role in the WIFS and with the conclusion that this now appears less likely. The implications of these findings will now be discussed in more detail.

Periods of Wheel-access

Considering the results of the experiments in this thesis and other previous studies, there is strong evidence to suggest that there is no relationship between how much rats' run and how much their food-consumption decreases after wheel-access. In Experiment 1, rats ran between 211-1175 and running in this range did not predict the size of the feeding-suppression. This finding is consistent with previous studies where with longer wheel-access there was also no relation between amount of running and the WIFS (Afonso & Eikelboom, 2003; Lattanzio & Eikelboom, 2003). It simply appears to be a matter of whether or not the rat has access to a running wheel and for how long. Previous research has showed only a slower development of the WIFS with 2 h wheel-access than 24 h wheel-access (Lattanzio & Eikelboom, 2003). The experiments in this thesis show not just a slower development of the WIFS with shorter wheel-

access but also a smaller magnitude of the feeding-suppression: longer wheel-access means a larger feeding-suppression.

The studies in this thesis suggest that the total duration of wheel-access the rat has experienced may be the most important factor in when the wheel-induced feeding-suppression develops. It may be that more time with the running-wheel allows for rats to become accustomed to the wheel's presence. In other words the wheel loses its novelty and it is at this point that the WIFS can be expressed. This is supported by the fact that rats with 24 h of wheel-access show a larger feeding-suppression on the first day of 3 h wheel-access than rats having their first experience with 3 h. The novelty hypothesis is supported by Experiment 2 where rats first introduced to the wheel for 3 h showed no feeding-suppression while rats that had previous 24 h wheel-access and then a no wheel period showed a feeding-suppression after their first experience with 3 h wheel-access. This hypothesis is also corroborated by a previous wheel-running experiment in which 24 h pre-exposure appeared sufficient to induce a WIFS with subsequent 3 h wheel-access (Adams et al., 2009). Experiment 4 showed that 6 h wheel-access is as efficient as 24 h wheel-access at inducing a WIFS. In the frame of the novelty explanation this finding would suggest that 6 h of wheel-access is sufficient for the wheel to lose novelty and therefore for rats to show a feeding-suppression.

Over all the experiments in this thesis, it appears that, after rats have become familiar with the wheel, the size of the WIFS is a function of the daily duration of wheel-access. The suppression with 24 h a day wheel exposure is always larger than that with 3 h a day wheel-access and in the fourth experiment 6 h of access produced a reliable feeding-suppression that fell in-between that seen with 3 and 24 h wheel-access. This is inconsistent with the results shown by Lattanzio & Eikelboom (2003) who found that 24 and 2 h wheel-access eventually

produced a similar feeding-suppression. Experiment 3 argues that this difference with short access is unlikely to be due to the timing of when in the day the wheel-access occurred. This suggests further studies systemically looking at the duration of wheel-access and the feeding-suppression is necessary.

The Temporal Profile of Used Drugs on the WIFS

In Experiment 4, it was found that neither URB597 nor THC at the selected doses were effective at reducing the size of the feeding-suppression. One possibility is that the doses were not high enough to reverse the WIFS – this could be addressed in future studies by using doses that are much higher, but it should be pointed out the doses used in Experiment 4 are similar to those used in previous work (Scherma et al., 2013). Future studies may wish to look at doses of THC similar to those used by Verty et al. (2011). Another possibility is that the timing of injection (after 6 h of wheel-access) may have been too late to prevent the WIFS. Previous studies have shown that the WIFS can be prevented after 3 h of wheel-access (Adams et al., 2009): suggesting 3 h may be a critical time to intervene. A possible study to be considered would be using the 6 h wheel-access paradigm from Experiment 4 and injecting rats in the middle of and after the 6 h period of wheel-access (every 3 h of wheel running). This would allow experimenters to determine the time course of the WIFS and if injections at different times have a greater impact.

Another way to determine the time course of the induction of the WIFS would be to take baseline feeding measurements from rats at fixed intervals through a 24 h period. Following this, rats would be introduced to a running-wheel for 6 h in the day time and food could be repeatedly weighed in the same manner as before. This could provide an index as to when rats decrease their food-consumption relative to introduction of the running-wheel. Researchers could then

reintroduce a running-wheel and inject rats at the time of (or just before) the maximum drop in food-consumption (or inject at a time so the drug will have reached peak effect by the point of maximum feeding-suppression). Alternatively, the injections could occur at various other times around wheel-access and around the timing of the feeding-suppression. Overall, measuring food-consumption in shorter intervals could allow researchers to more accurate track the WIFS and potentially determine the ideal drug administration time to reverse it.

Another possibility is to give rats 24 h of wheel-access and note the time at which food-consumption decreases in a 24 h paradigm through hourly (or otherwise) measurements of food. Then the drug could be administered prior to the feeding-suppression and one could see if the suppression was prevented. This way, researchers would benefit from the robust suppression from 24 h of wheel-access and also be able to attempt to prevent the feeding-suppression when it begins.

Diet and the WIFS

Verty et al. (2011) suggested that diet may be important in determining the effect of cannabinoids on rats' food-consumption following wheel-access. Their study showed that THC was most able to restore rats' weight when a high-fat diet was available. Normal chow and THC showed the smallest attenuation of weight-loss. In terms of the WIFS, wheel-access alone is well documented to decreased consumption of regular chow and most likely is capable of decreasing consumption of a high-fat diet. A study by Satvat and Eikelboom (2006) suggest that highly palatable food is affected by wheel-access more than normal chow. In their study they provided rats with 24 h wheel-access and observed that the consumption of a familiar sucrose solution decreased much more than chow consumption following this wheel-access. The WIFS was larger for sucrose solutions than for chow. Future studies should therefore incorporate another group of

rats that receive a high-fat diet into a design similar to Experiment 4. Furthermore, a URB597 dose of 0.3 mg/kg and a THC dose of either 0.125 or 0.25 mg/kg (i.e. the same THC doses used in Experiment 4) were shown to be effective in increasing consumption of margarine (i.e. a highly palatable food source) relative to normal chow (Scherma et al., 2013) – therefore those doses may be viable to reverse the WIFS.

Another possibility for future research would be to make both a regular diet and high-fat diet available to each rat. Following wheel-introduction, researchers would be able to examine any shift in preference that occurs. It could also be examined whether or not consumption of the high-fat diet decreases more than the regular diet or vice versa. This could lead to testing THC or URB597 to see if the rats' original preference for each diet is restored. These findings would then mirror a human study that showed exercise can cause a shift in preference towards healthier foods away from less healthy options (Killgore et al., 2013). Research paradigms that use more than one type of diet with animals would reflect options available to humans: we generally have variety of food available. There are healthier alternatives (i.e. the regular chow for rats) and then there are the less healthy, more palatable alternatives (i.e. the high-fat diet for rats). Therefore, a study using more than one type of diet and some dose of the drug would be interesting in expanding the existing WIFS effect and understanding specifically how neurological agents affect it.

Other Systems to Explore in the WIFS

Since agents affecting the cannabinoid system were used in Experiment 4, it may be interesting to explore specific receptor subtypes of the cannabinoid system (CB1 or CB2) and if each has a role in the WIFS. This could be done in many ways. Researchers could utilize animal mutations lacking a receptor subtype (i.e. CB1 knock-out mice) and see if the WIFS persists and

is comparable to that found in wild-type animals. It may also be interesting to explore the role of a CB1 antagonist/inverse agonist such as rimonabant. A drug like rimonabant is well documented to decrease food-consumption (i.e. Scherma et al., 2013) and therefore should exacerbate the WIFS. The ECS also has connections to other systems that are of interest to the WIFS. Particularly, it has been speculated that corticotrophin releasing factor (CRF) may be altered by wheel-access (Lattanzio & Eikelboom, 2003) and it has also been shown that leptin is increased following wheel-access (Shapiro et al., 2011).

It was previously hypothesized that wheel-access may increase CRF and therefore increase levels of stress in rats (Lattanzio & Eikelboom, 2003). That the wheel is a stressful stimulus is supported by the fact that it is capable of suppressing food-consumption – similar to other anxiety assessments (i.e. Bambico, Nguyen, Katz, & Gobbi, 2010). Furthermore wheel-access greatly impacts consumption of a novel sucrose solution (Satvat & Eikelboom, 2006). It would be interesting for future studies to directly compare the effect of wheel-access on food-consumption in the context of an anxiety assessments (like the novelty induced hypophagia assay) on food-consumption (Bambico et al., 2010; Gamble-George et al., 2013). If stress is increased following wheel-access future studies may also seek to measure levels of stress from blood plasma (i.e. corticosterone) and may choose to test anxiolytic agents as a way to block the WIFS.

The final possibility for exploration is leptin. Leptin has been shown to be increased by wheel-access and this elevation in leptin may be responsible for the decrease in food-consumption seen in the WIFS paradigm (Shapiro et al., 2011). Future studies could incorporate administrations of leptin to see how it impacts the WIFS effect. Low leptin levels have also been tied to increased running and therefore administration of leptin to decrease the WIFS via

exercise reduction is another possibility (Exner et al., 2000). Leptin is an interesting prospect for future research as there appears to be a complex relationship between leptin, exercise, and food-consumption.

Final Summary of Findings and Future Prospects in WIFS Research

The first three experiments sought to explain the lack of a WIFS with limited wheel-access found in previous work (Peckham et al., 2013): specifically to determine what controlled the expression of the WIFS and why it was not always evident. In the first experiment looking for factors controlling the WIFS found no predictors in any of the measurements taken. That is, wheel-turns and body-weight were not related to the size of the feeding-suppression that results from unlocked wheel-access. Furthermore, it appears that more than one day of wheel-access is necessary to generate a WIFS and therefore future drug work may wish to target the second or third day of unlocked wheel-access rather than the first to look for a WIFS. In other words, the second or third day of wheel-access should be the day of feeding-suppression that is targeted to be 'prevented'. Not only this, Experiment 3 and Experiment 4 show that the days of unlocked wheel-access can be separated and this suppression will still occur. Experiment 4 suggests that 6 h of wheel-access is a reasonable amount of time in the running-wheel for the WIFS to be consistently expressed. In sum, the 4 experiments reported presently suggest that future studies should use a WIFS paradigm that uses 6 h for each wheel-access period and that at least on 6 h wheel-access period should occur before a test day (i.e. before a drug is tested).

Second, at least for a first assessment, it appears that the WIFS is not controlled by reductions in the endocannabinoids as attempts to elevate the activity of this system did not change the WIFS. Future studies should seek to understand the time-course of the WIFS (i.e. when does food-consumption begin to decrease relative to the period of wheel-access), how

different diets are affected by the WIFS, how leptin influences the WIFS, how CRF is influenced by the WIFS, and how other cannabinoids and CB1 receptors specifically control the WIFS (if at all).

With these prospects presented, it seems that this research has successfully developed a WIFS paradigm, which can be used in future, studies. Though no drug effects were seen in Experiment 4, it may be useful in determining the effectiveness of various drugs in restoring food-consumption to normal levels. Taking this model forward could be a step in understanding the mechanisms that make rats unable to regulate energy output and intake in an optimal manner. This has potential to aid in the understanding of AN and potentially help AN patients to consume normal amounts of food and reduce the drive to exercise to extreme levels. This end is in the distant future, however, the WIFS model developed through these experiments may be a means to progress. Overall, this work has developed a working WIFS paradigm that – though much is left to understand about the mechanism of WIFS – may lead to insights into AN, which may then lead to proper treatment of this disorder.

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Table 1. Mean (±SEM) Baseline Food-Consumption, Food-Consumption, Feeding-Suppression, and Wheel-Turns of rats in Experiment 1.

		Day 5/15	Day 6/16	Day 7/17	Day 8/18
24 h	4 Day Baseline Average	32.89 (±0.39)	--	--	--
	Food-Consumption	27.14 (±0.56)	24.77 (±0.52)	25.07 (±0.67)	25.08 (±0.57)
	Feeding-Suppression	-5.76 (±0.61)	-8.13 (±0.49)	-7.83 (±0.65)	-7.82 (±0.62)
	Wheel-Turns	605 (±30)	365 (±21)	308 (±20)	331 (±23)
3 h	4 Day Baseline Average	32.16 (±0.44)	--	--	--
	Food-Consumption	30.07 (±0.59)	30.75 (±0.51)	28.78 (±0.53)	30.9 (±0.54)
	Feeding-Suppression	-2.09 (±0.49)	-1.41 (±0.48)	-3.38 (±0.51)	-1.18 (±0.48)
	Wheel-Turns	133 (±11)	124 (±9)	107 (±8)	112 (±16)

Notes: All rows but 'Wheel-Turns' are measured in grams (g). Data reported in the rows of 24 h (Days 5, 6, 7, 8) are for the same rats as reported in the 3 h (Days 15, 16, 17, 18) rows. The rows show the average of food-consumption in the 4 days preceding wheel-access ('4 Day Baseline Average'), how much rats ate following wheel-access ('Food-Consumption'), the difference between Food-Consumption and 4 Day Baseline Average ('Feeding-Suppression'), and the total number of wheel-turns ('Wheel-Turns').

Table 2. Correlations of Wheel-Turns and Body-Weight to the WIFS (Experiment 1: 24 h wheel-access).

		Day 5	Day 6	Day 7	Day 8
Wheel-turns	Pearson r	.067	.131	.016	.092
	N	62	61	61	59
	P-value	.607	.314	.9	.489
Body Weight	Pearson r	-.251	-.111	-.116	0.231
	N	63	63	63	63
	P-value	.047	.387	.365	.068

Notes: Pearson product moment correlations for the relationship between the feeding-suppression following each period of 24 h wheel-access and the number of wheel-turns ('Wheel-Turns'). Similar correlations are shown between the feeding-suppression and rats' body weight in grams (g; 'Body Weight').

Table 3. Correlations of Wheel-Turns and Body-Weight to the WIFS (Experiment 1: 3 h wheel-access).

		Day 15	Day 16	Day 17	Day 18
Wheel-turns	Pearson r	.071	.047	-.098	.291
	N	63	61	61	61
	P-value	.581	.721	.452	.023
Body Weight	Pearson r	-.071	-.026	-.044	-.096
	N	62	62	62	61
	P-value	.582	.844	.732	.461

Notes: Pearson product moment correlations between the feeding-suppression following each period of 3 h wheel-access and the number of wheel-turns ('Wheel-Turns'). Correlations are also shown between the feeding-suppression and rats' body weight in grams (g; 'Body Weight').

Table 4. Correlations between wheel-turns during each day of 24 h wheel-access and wheel-turns during each day of 3 h wheel-access. (Experiment 1).

		Day 15 (3 h)	Day 16 (3 h)	Day 17 (3 h)	Day 18 (3 h)
Day 5 (24 h)	Pearson r	.620	.559	.687	.477
	N	62	60	60	61
	P-value	<.001	<.001	<.001	<.001
Day 6 (24 h)	Pearson r	.601	.683	.542	.441
	N	61	59	59	60
	P-value	<.001	<.001	<.001	<.001
Day 7 (24 h)	Pearson r	.478	.547	.515	.429
	N	61	59	59	60
	P-value	<.001	<.001	<.001	.001
Day 8 (24 h)	Pearson r	.557	.611	.582	.416
	N	59	58	58	59
	P-value	<.001	<.001	<.001	.001

Table 5. Correlations between the feeding-suppression following each day of 24 h wheel-access and the feeding-suppression following each day of 3 h wheel-access (Experiment 1).

		Day 15 (3 h)	Day 16 (3 h)	Day 17 (3 h)	Day 18 (3 h)
Day 5 (24 h)	Pearson r	.148	.198	.085	.160
	N	63	63	63	62
	P-value	.247	.119	.507	.215
Day 6 (24 h)	Pearson r	.242	.315	.239	.244
	N	63	63	63	62
	P-value	.056	.012	.060	.056
Day 7 (24 h)	Pearson r	.137	.227	.008	.204
	N	63	63	63	62
	P-value	.283	.074	.953	.112
Day 8 (24 h)	Pearson r	.149	.437	.193	.345
	N	63	63	63	62
	P-value	.245	<.001	.13	.006

Table 6. Mean (±SEM) Baseline Food-Consumption, Food-Consumption, Feeding-Suppression, and Wheel-Turns of rats in Experiment 2.

		Day 4/14	Day 5/15	Day 6/16	Day 7/17
24 h	Baseline Average	34.25 (±0.54)	--	--	--
	Food-Consumption	26.6 (±0.9)	26.17 (±0.88)	25.13 (±0.94)	26.89 (±1.01)
	Feeding-Suppression	-7.65 (±0.92)	-8.08 (±0.87)	-9.12 (±1.08)	-7.36 (±1.01)
	Wheel-Turns	958 (±67)	641 (±47)	563 (±38)	587 (±66)
3 h	Baseline Average	33.61 (±0.55)	--	--	--
	Food-Consumption	31.92 (±0.87)	30.21 (±0.63)	31.16 (±1.3)	29.39 (±0.62)
	Feeding-Suppression	-1.69 (±0.69)	-3.4 (±0.65)	-2.45 (±1.41)	-4.22 (±0.59)
	Wheel-Turns	290 (±20)	290 (±24)	267 (±26)	265 (±25)

Notes: All rows but 'Wheel-Turns' are measured in grams (g). Rows, in order, show the 4 day Baseline food-consumption, the food-consumption following wheel-access, the change in feeding from baseline, and the number of wheel-turns of rats used in Experiment 2.

Table 7. Mean (±SEM) Baseline Food-Consumption, Food-Consumption, Feeding-Suppression, and Wheel-Turns of rats in Experiment 3.

		Day 4	Day 7	Day 10	Day 13
Early	2 day Baseline Average	33.13 (±0.97)	34.19 (±1)	35.42 (±0.95)	34.56 (±0.83)
	Food-Consumption	31.71 (±0.82)	31.02 (±1.36)	31.27 (±0.76)	30.56 (±0.8)
	Feeding-Suppression	-1.42 (±0.61)	-3.17 (±1.18)	-4.15 (±0.83)	-4 (±0.82)
	Wheel-Turns	312 (±32)	411 (±48)	564 (±66)	575 (±69)
Late	2 day Baseline Average	34.56 (±0.69)	33.83 (±0.96)	35.63 (±1.02)	34.38 (±0.97)
	Food-Consumption	33.75 (±1.26)	30.81 (±0.98)	31.49 (±1.41)	32.69 (±1.38)
	Feeding-Suppression	-0.81 (±1.03)	-3.01 (±1.09)	-4.13 (±2.01)	-1.69 (±1.64)
	Wheel-Turns	213 (±26)	237 (±36)	280 (±38)	270 (±44)

Notes: Rows labeled ‘Wheel-Turns’ are the only rows not measured in grams (g). Rows, in order, show the 2 day Baseline food-consumption, the food-consumption following wheel-access, the change in feeding from baseline, and the number of wheel-turns of rats used in Experiment 3.

Table 8. Mean (±SEM) Baseline Food-Consumption, Food-Consumption, Feeding-Suppression, and Wheel-Turns of rats in Experiment 4a.

URB597	Vehicle		Low		Middle		High	
	Locked	Open	Locked	Open	Locked	Open	Locked	Open
2 Day Baseline Average	32.59 (±078)	32.45 (±0.85)	32.36 (±0.93)	33.33 (±0.78)	31.8 (±0.78)	32.59 (±0.79)	32.53 (±0.7)	33.68 (±0.76)
24 h Food-Consumption	32.01 (±1.09)	27.85 (±1.12)	30.7 (±0.59)	29.37 (±0.7)	31.91 (±0.78)	28.04 (±1.11)	31.47 (±0.98)	28.27 (±1.08)
Feeding-Suppression	-0.58 (±1.04)	-4.6 (±0.91)	-1.66 (±0.72)	-3.95 (±0.58)	0.11 (±0.69)	-4.55 (±0.93)	-1.06 (±0.64)	-5.4 (±1.05)
Wheel-Turns	--	585 (±102)	--	520 (±75)	--	688 (±148)	--	628 (±114)

Notes: All rows except for 'Wheel-Turns' were measured in grams (g). Rows, in order, show 2 day baseline food-consumption, food-consumption following wheel-access, feeding-suppression, and wheel-turns for the 14 rats used in Experiment 4a on each testing day.

Table 9. Mean (±SEM) Baseline Food-Consumption, Food-Consumption, Feeding-Suppression, and Wheel-Turns of rats in Experiment 4b.

THC	Vehicle		Low		High	
	Locked	Open	Locked	Open	Locked	Open
2 Day Baseline Average	33.36 (±0.98)	33.41 (±0.86)	32.97 (±1.19)	33.53 (±0.84)	33.36 (±1.19)	32.93 (±1.07)
24 h Food-Consumption	32.91 (±0.98)	31.05 (±0.97)	32.66 (±0.99)	30.07 (±0.92)	32.47 (±1.11)	28.87 (±1.27)
Feeding-Suppression	-0.046 (±0.71)	-2.36 (±0.64)	-0.31 (±0.32)	-3.46 (±0.68)	-0.89 (±0.75)	-4.06 (±0.68)
Wheel-Turns	--	453 (±43)	--	411 (±44)	--	450 (±60)

Notes: All rows except for 'Wheel-Turns' were measured in grams (g). Rows, in order, show 2 day baseline food-consumption, food-consumption following wheel-access, feeding-suppression, and wheel-turns for the 15 rats used in Experiment 4b on each testing day.

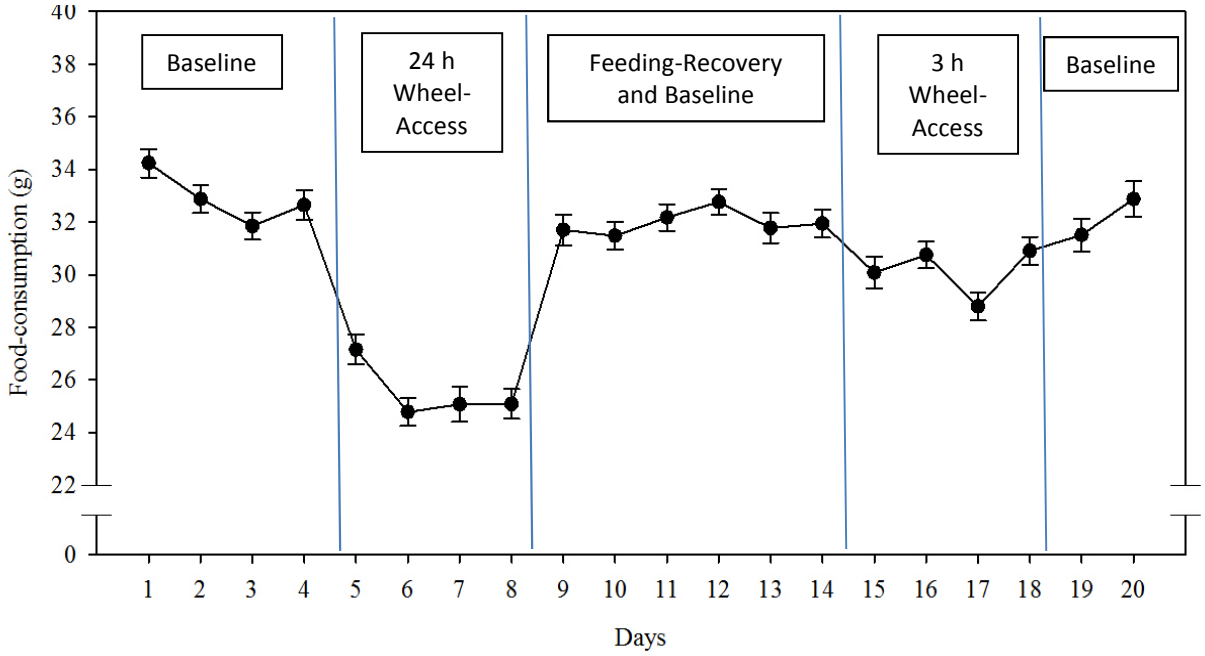

Figure 1. Mean (±SEM) food-consumption over the days Experiment 1. On days 4, 5, 6, and 7 rats were given 24 h of wheel-access. Therefore Days 5, 6, 7, and 8 represent days where the feeding-suppression was present. Similarly, Days 14, 15, 16, and 17 were days on which rats received 3 h of wheel-access. Days 15, 16, 17, and 18 show food-consumption on days when the feeding-suppression would be evident. This graph illustrates the fact that 24 h of wheel-access seems to induce a larger feeding-suppression than 3 h of wheel-access.

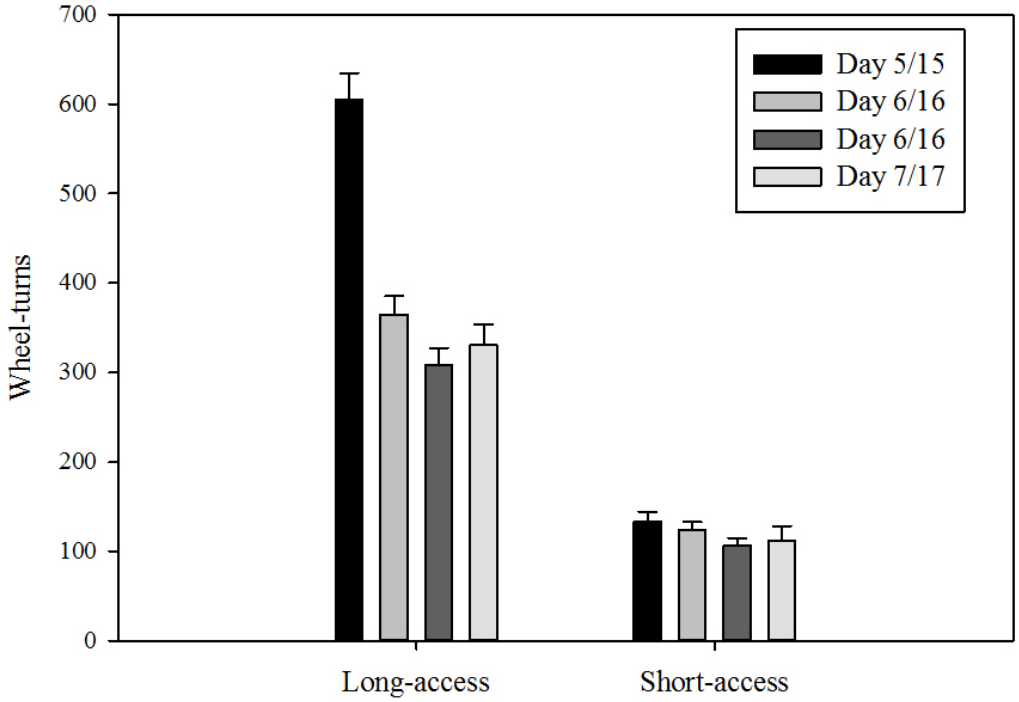

Figure 2. Mean (±SEM) wheel turns over days of Experiment 1. When rats were given 24 h of wheel-access (Long-access) running was higher on the first day. When rats were subsequently given 3 h of wheel-access running was constant across days. Running was higher with 24 than 3h wheel-access.

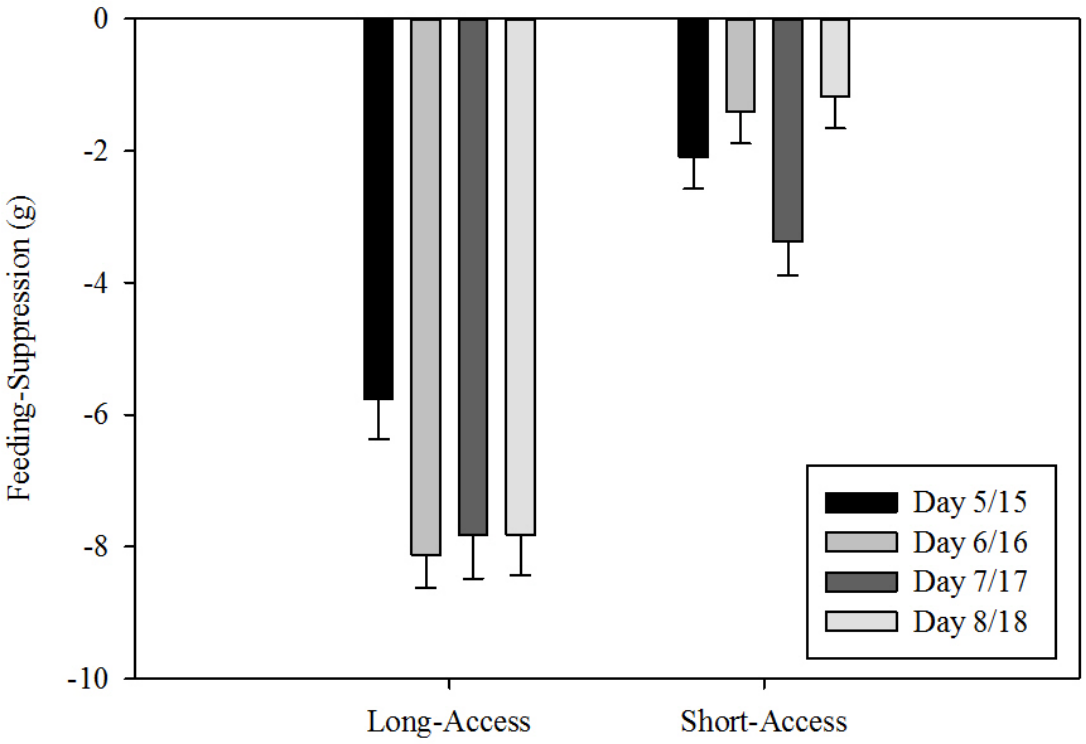

Figure 3. Mean (±SEM) drops in food-consumption relative to average baseline feeding in Experiment 1. Groups of bars show the feeding-suppression following days of 24 h wheel-access (Long-Access) and 3 h wheel-access (Short-Access).

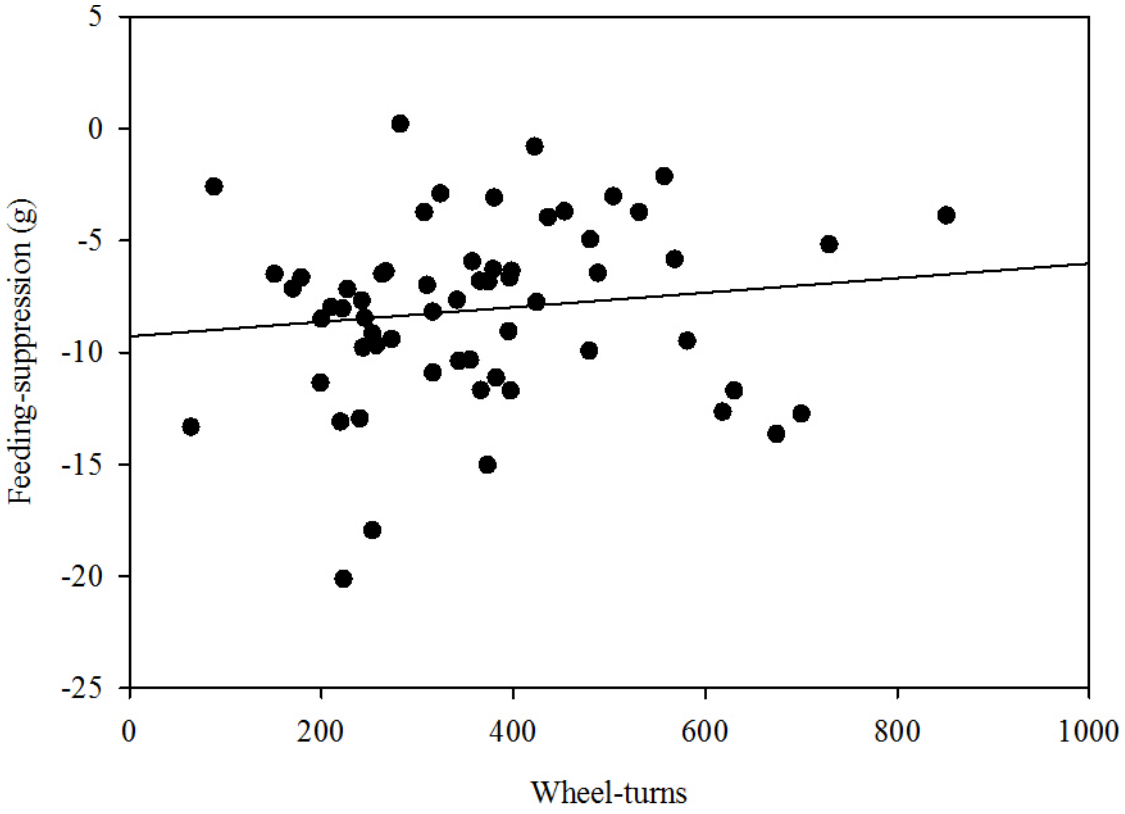

Figure 4. Relationship between wheel-turns and the feeding-suppression with 24 h wheel-access (Experiment 1). Regression is for Day 6 (i.e. the day of the largest feeding-suppression for 24 h wheel-access).

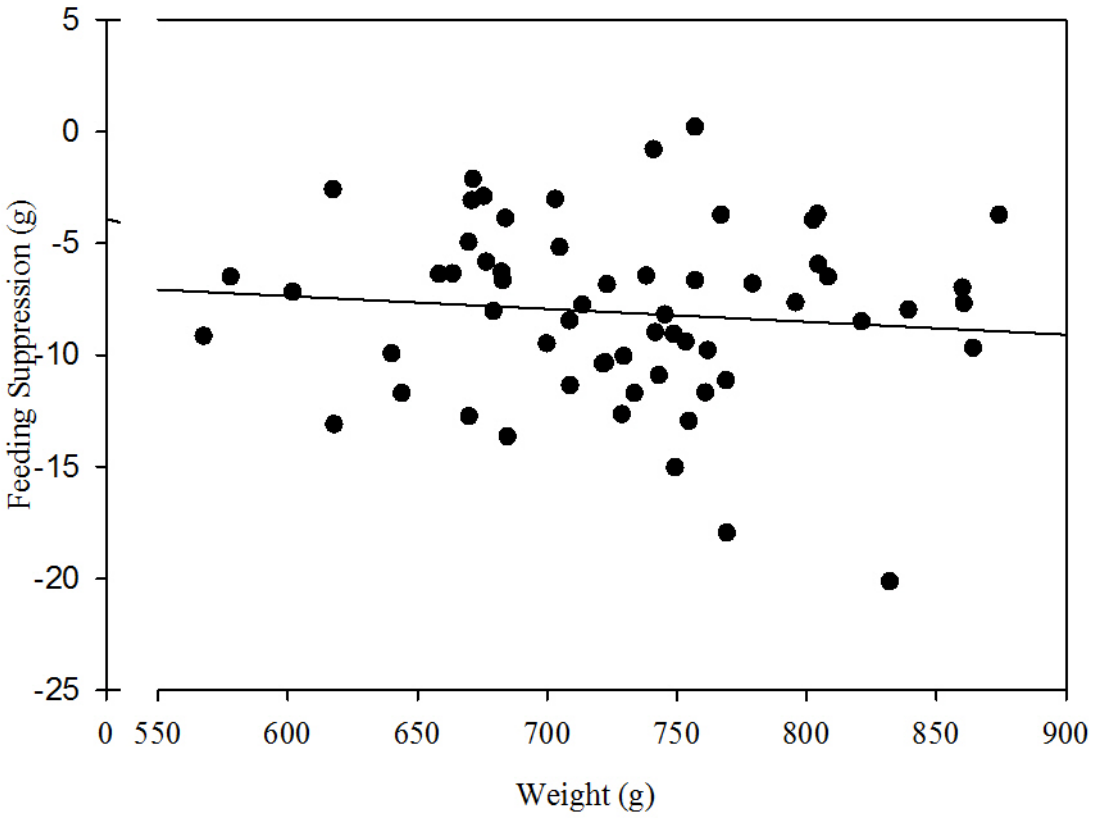

Figure 5. Relationship between rats' weights and the feeding-suppression with 24 h wheel-access (Experiment 1). Regression is for Day 6 (i.e. the day of the largest feeding-suppression for 24 h wheel-access).

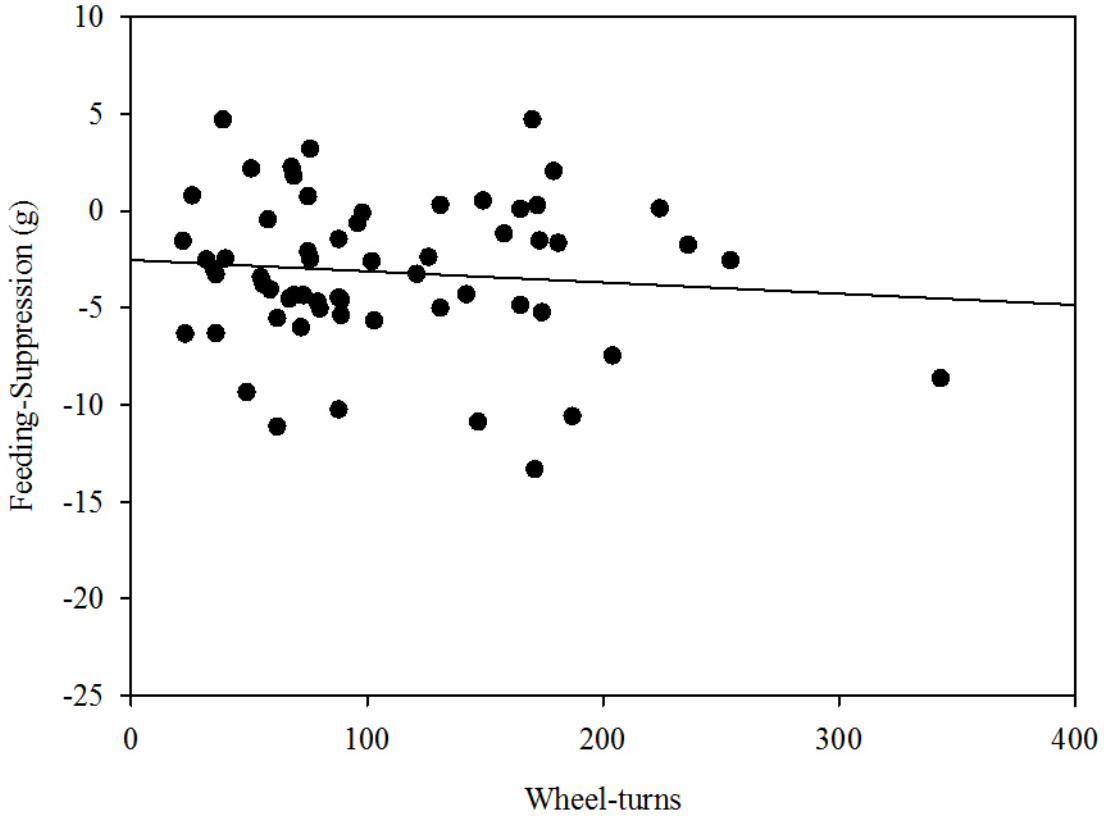

Figure 6. Relationship between wheel-turns and the feeding-suppression with 3 h wheel-access (Experiment 1). Regression is for Day 17 (i.e. the day of the largest feeding-suppression for 3 h wheel-access). Note the scale change for wheel turns in comparison to Figure 4.

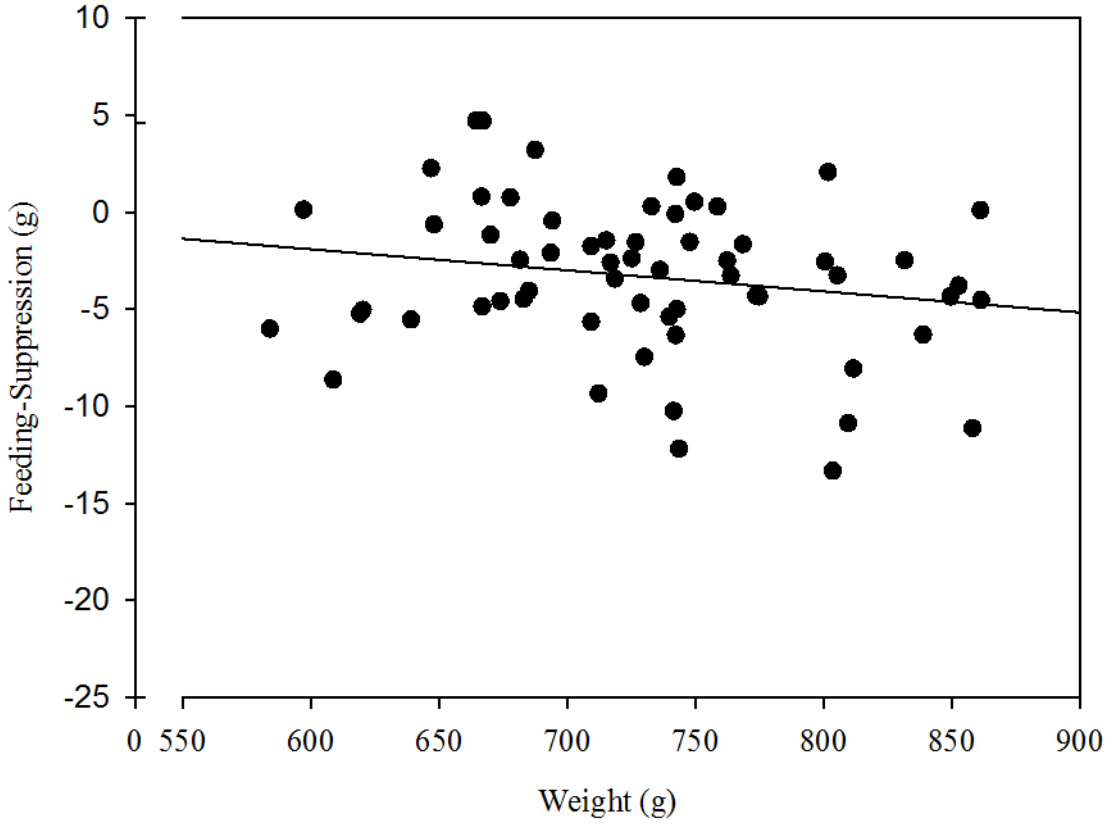

Figure 7. Relationship between rats' weights and the feeding-suppression with 3 h wheel-access (Experiment 1). Regression is for Day 17 (i.e. the day of the largest feeding-suppression for 3 h wheel-access).

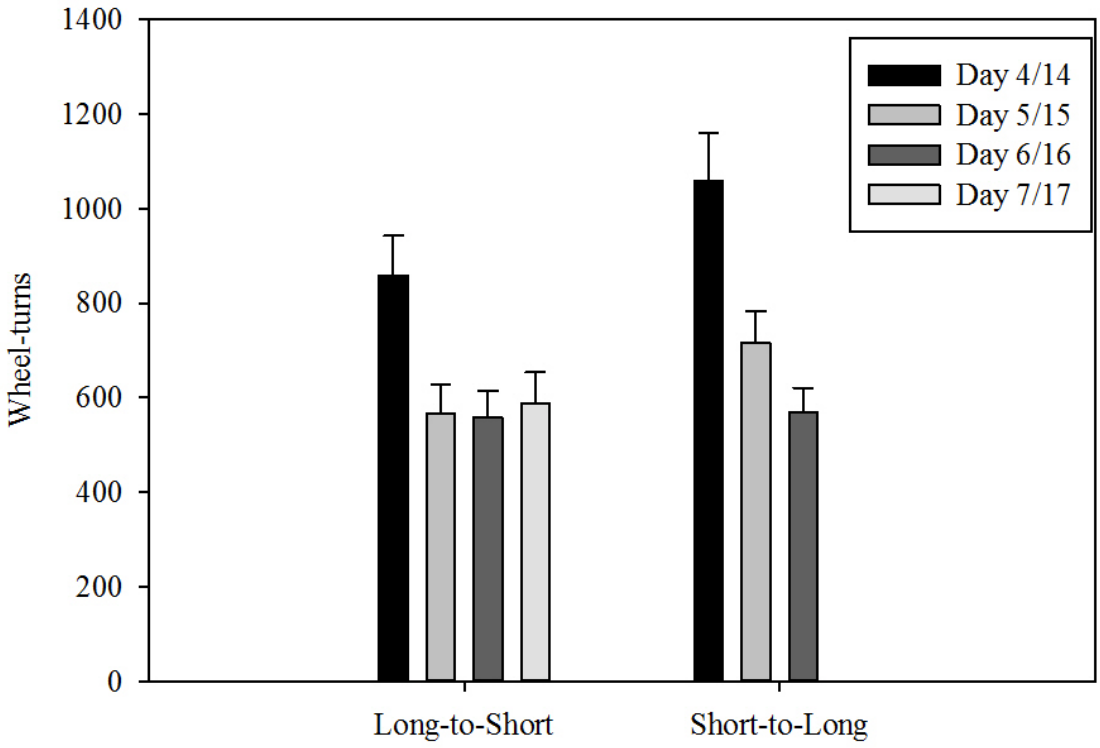

Figure 8. Mean (±SEM) for both groups in Experiment 2 (24 h wheel-access). For the Long-to-Short group these data are for Days 4-7 whereas for the Short-to-Long group these data are for Days 14-17. The fourth day (Day 17) of data for the Short-to-Long group is missing due to technical errors.

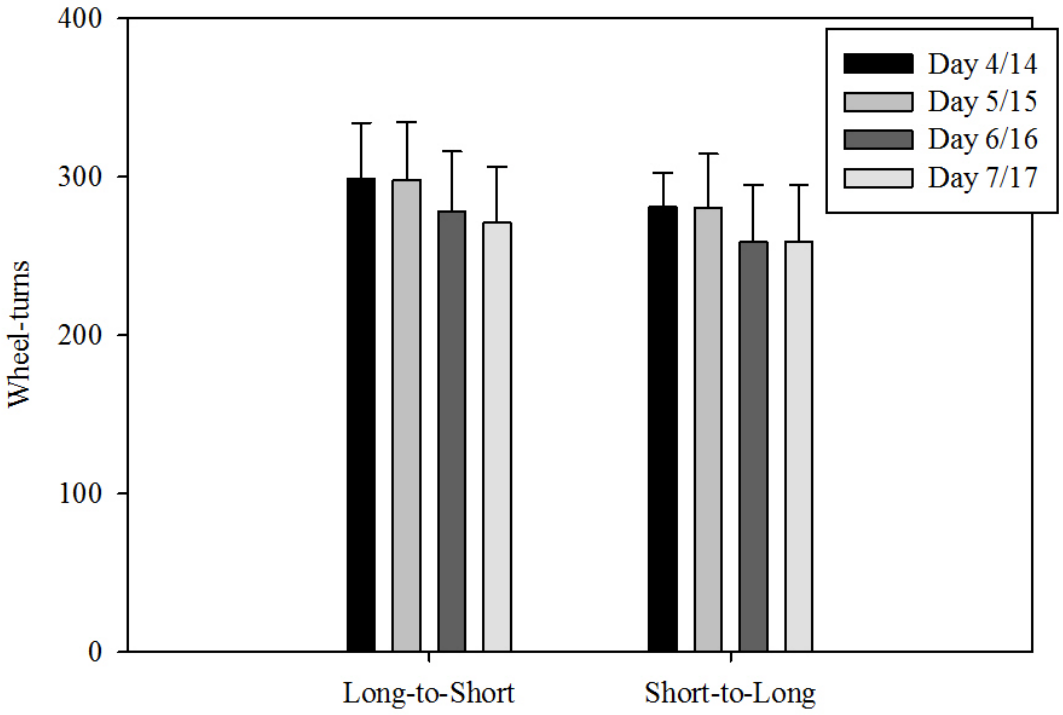

Figure 9. Mean (±SEM) number of wheel-turns for both groups in Experiment 2 (3 h wheel-access). For the Long-to-Short group these data are for Days 14-17 whereas for the Short-to-Long group these data are for Days 4-7.

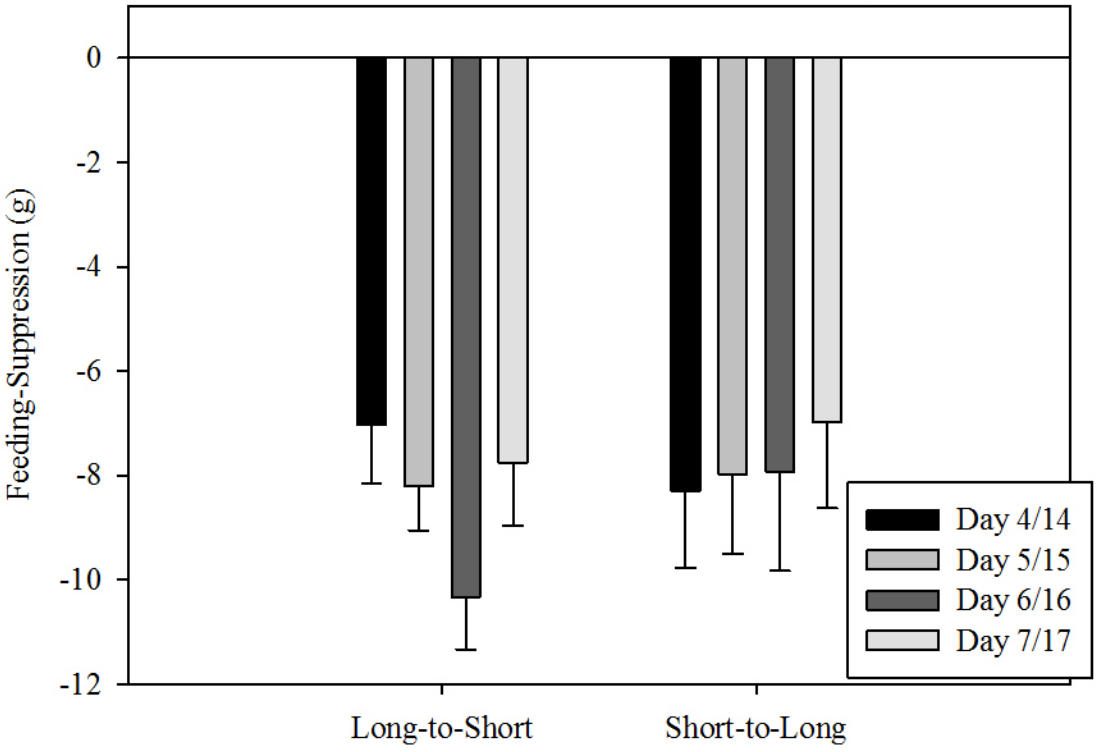

Figure 10. Mean (±SEM) drops in food-consumption relative to average baseline feeding for both groups in Experiment 2 (24 h wheel-access). For the Long-to-Short group these data are for Days 4-7 whereas for the Short-to-Long group these data are for Days 14-17.

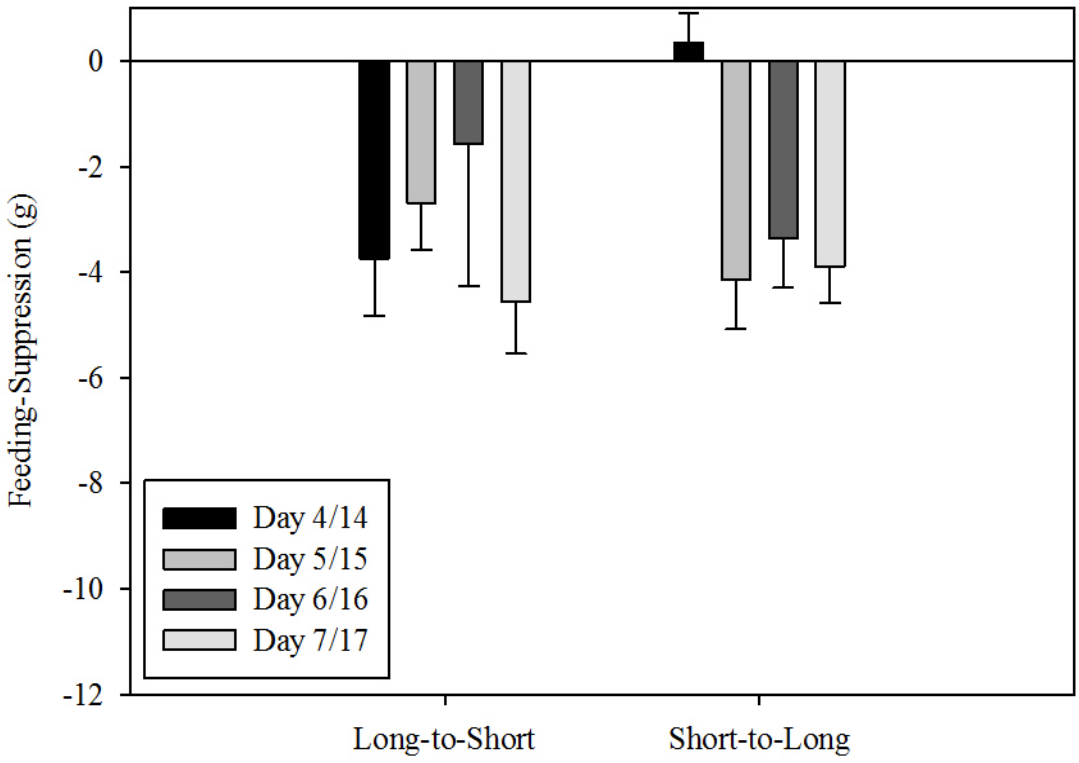

Figure 11. Mean (±SEM) drops in food-consumption relative to average baseline feeding for both groups in Experiment 2 (3 h wheel-access). For the Long-to-Short group these data are for Days 14-17 whereas for the Short-to-Long group these data are for Days 4-7.

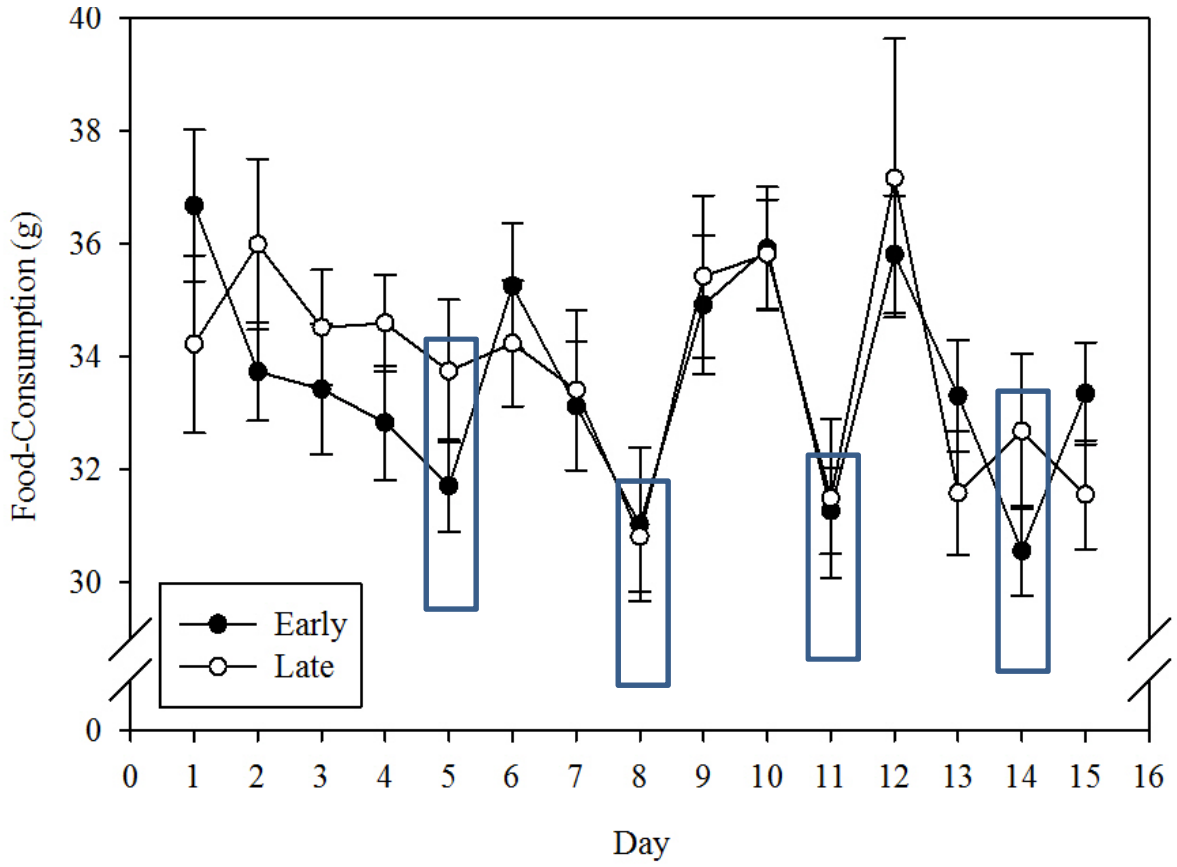

Figure 12. Mean (±SEM) food-consumption by both groups of rats across Experiment 3. Filled circles represent data from the 'Early' group whereas empty circles represent data from the 'Late'3 h of wheel-access was given to rats on Day 4, 7, 10, and 13. Therefore Day 5, 8, 11, and 14 show food-consumption in which the feeding-suppression should be evident. Boxes have been placed over these days' data points to highlight these data.

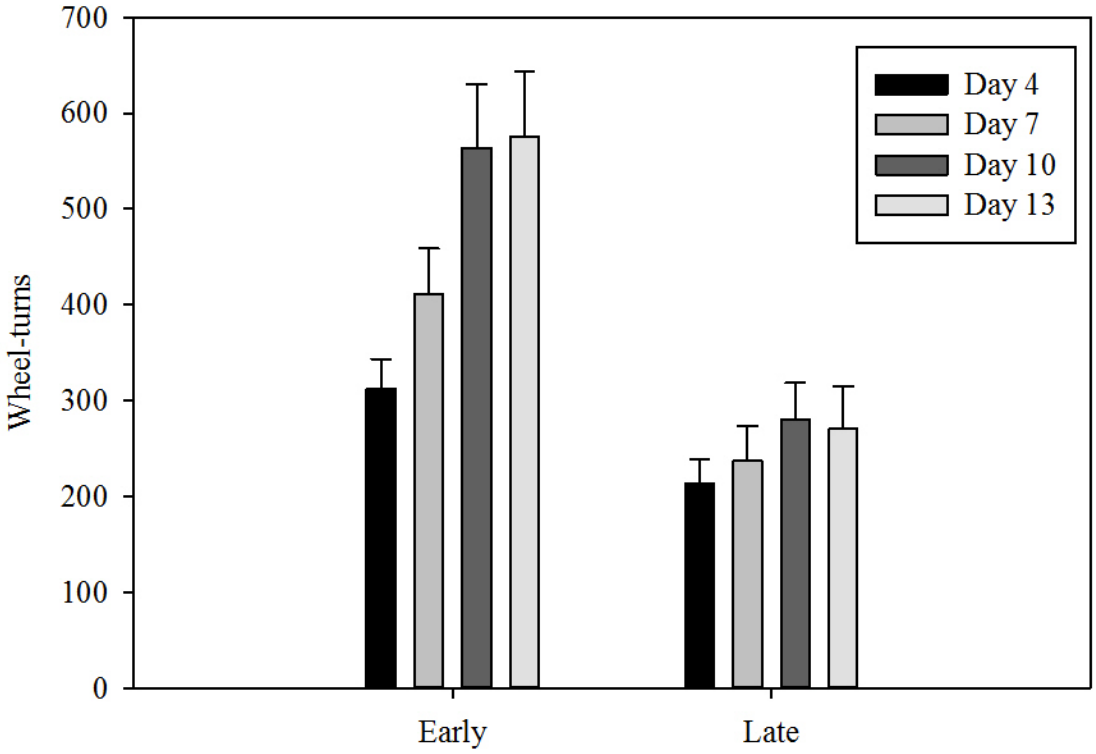

Figure 13. Mean (±SEM) wheel-turns by both groups of rats in Experiment 3 'Early' refers to the rats that got the wheel early in their light cycle (Early: in wheels at 11:30 h and out at 14:30 h) and 'Late' refers to the rats that got the wheel late in their light cycle (Late: in wheels at 17:30 h and out at 20:30 h). Bars within each cluster show wheel-turns on a given day of wheel-access. Rats that received the wheel earlier in their light cycle showed overall higher levels of running than rats that received the wheel later in their light cycle.

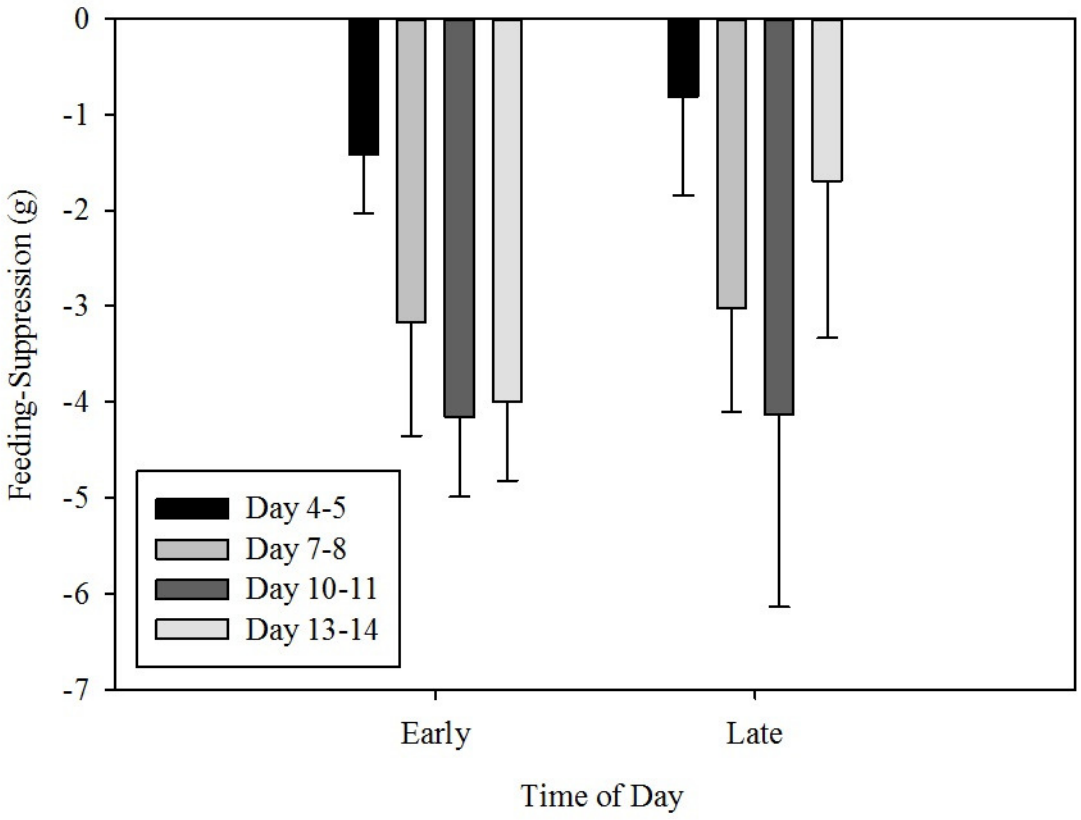

Figure 14. Mean (±SEM) feeding-suppression for each day of 3 h wheel-access in Experiment 3. This graph shows the most robust feeding-suppression occurs on the third day of wheel-access (Day 11).

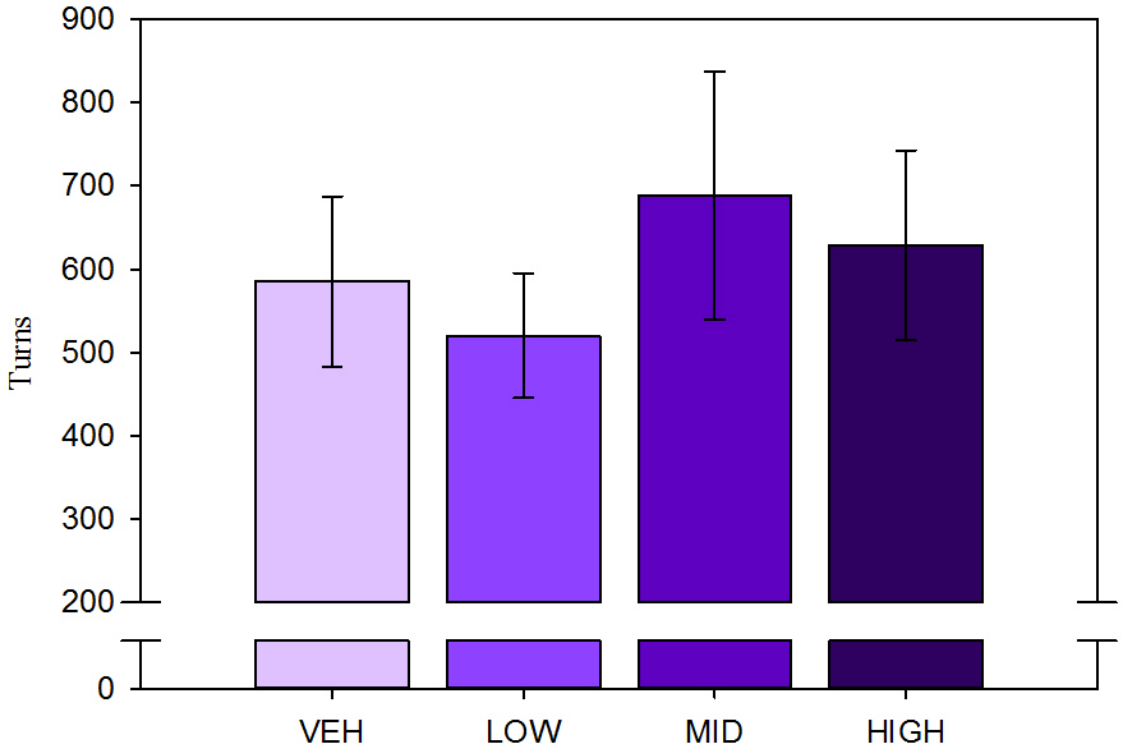

Figure 15. Mean (±SEM) wheel-turns for rats in Experiment 4a. Rats did not differ significantly in their wheel running regardless of a later injection of 0 ('VEH'), 0.17 ('LOW'), 0.5 ('MID'), or 1.0 mg/kg ('HIGH') of URB597.

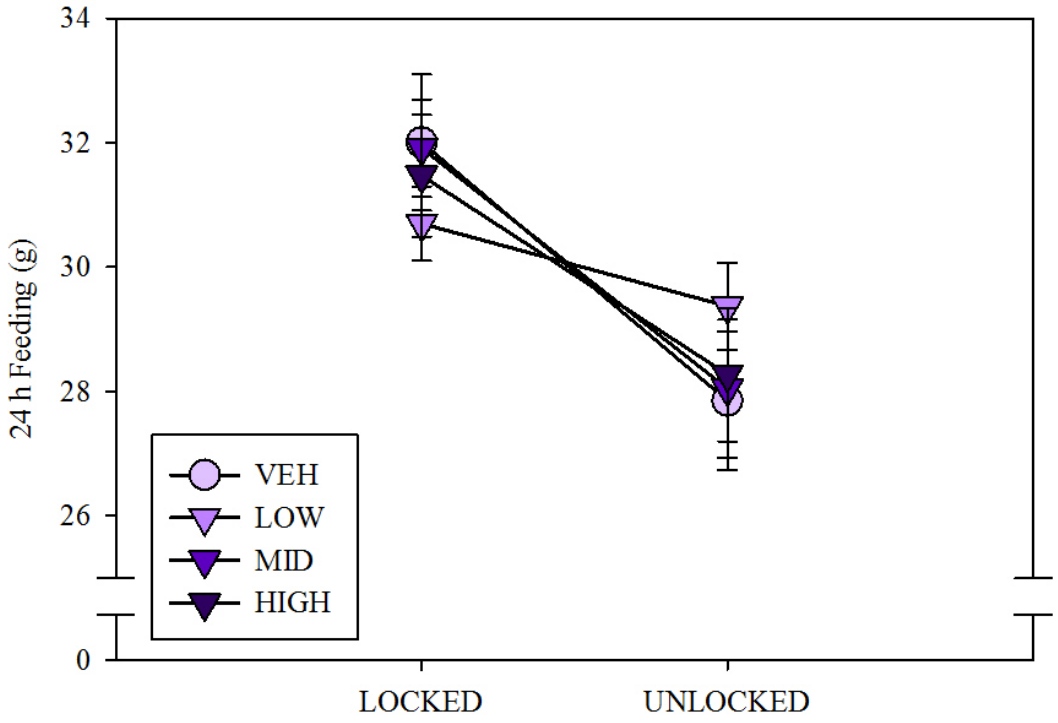

Figure 16. Mean (±SEM) food-consumption in grams for rats in Experiment 4a. Points over 'Locked' show 24 h food-consumption after 6 h of locked wheel-access. Points over 'Unlocked' show food-consumption after 6 h of unlocked wheel-access. Each point represents the same 14 rats following a 0 ('VEH'), 0.17 ('LOW'), 0.5 ('MID'), or 1.0 mg/kg ('HIGH') injection of URB597.

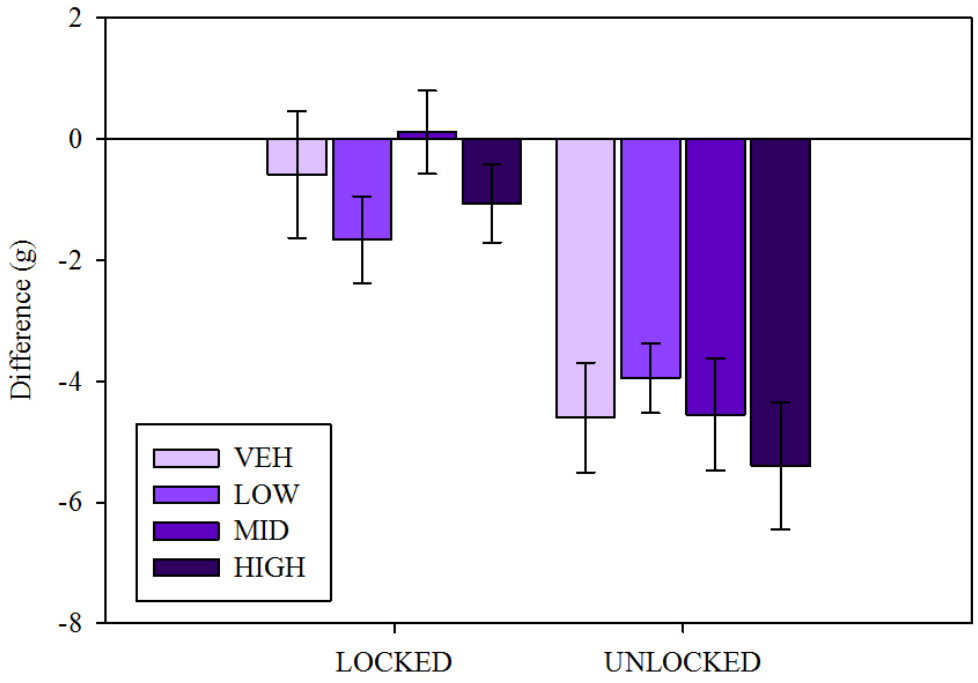

Figure 17. Mean (±SEM) feeding-suppression for rats in Experiment 4a. Data collected following 6 h periods of wheel-access (either 'Locked' or 'Unlocked') and i.p. injections of 0 ('VEH'), 0.17 ('LOW'), 0.5 ('MID'), or 1.0 mg/kg ('HIGH') URB597 doses. Differences are from baseline (2 days prior to wheel-access) food-consumption.

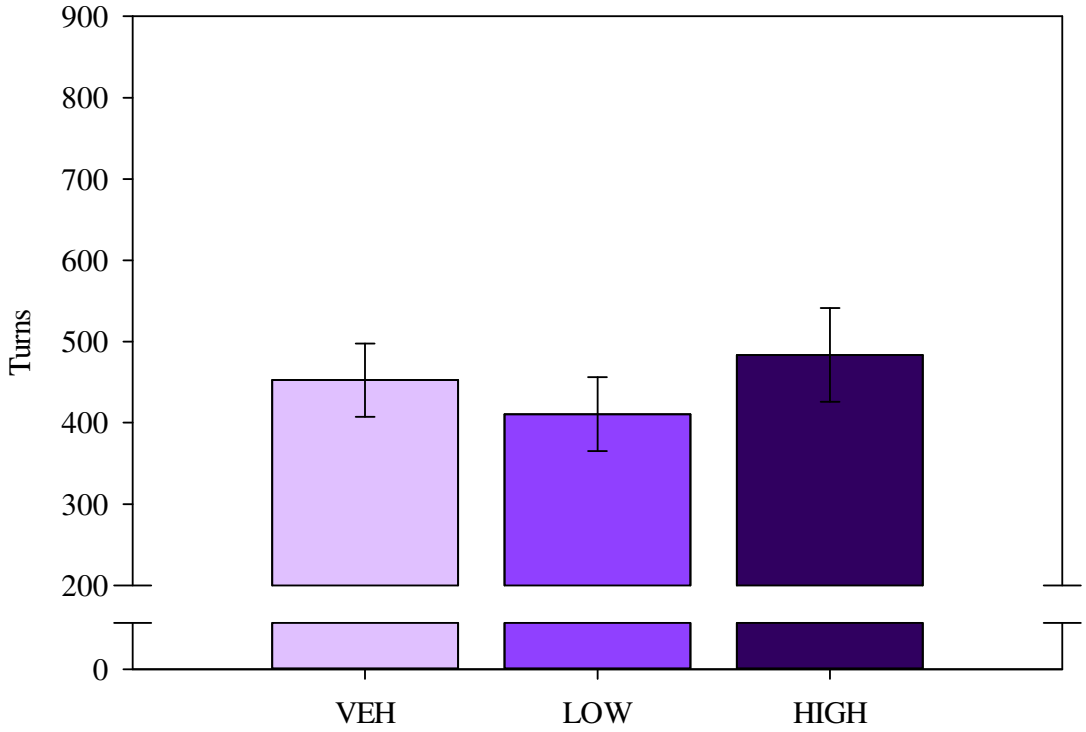

Figure 18. Mean (±SEM) wheel-turns for rats in Experiment 4b. Rats did not differ in the number of wheel-turns regardless of a later injection 0 ('VEH'), 0.125 ('LOW'), or 0.25 mg/kg ('HIGH') of THC.

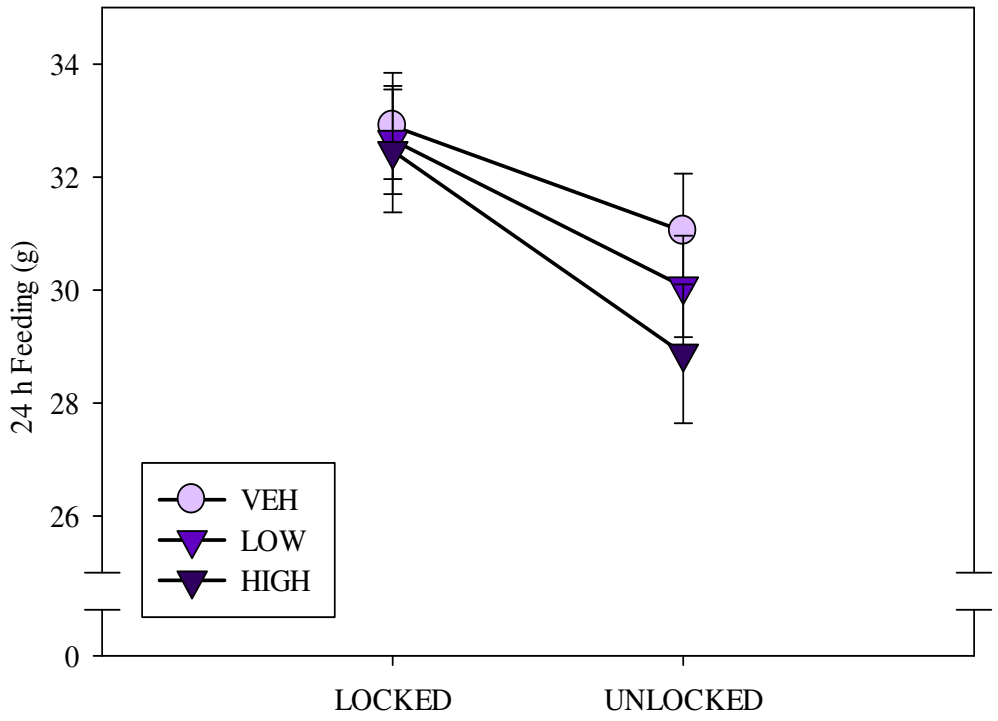

Figure 19. Mean (±SEM) food-consumption in grams for rats in Experiment 4b. Points over 'Locked' show food-consumption after 6 h of locked wheel-access. Points over 'Unlocked' show food-consumption after 6 h of unlocked wheel-access. Each point represents the same 15 rats following a 0 ('VEH'), 0.125 ('LOW'), or 0.25 mg/kg ('HIGH') of THC.

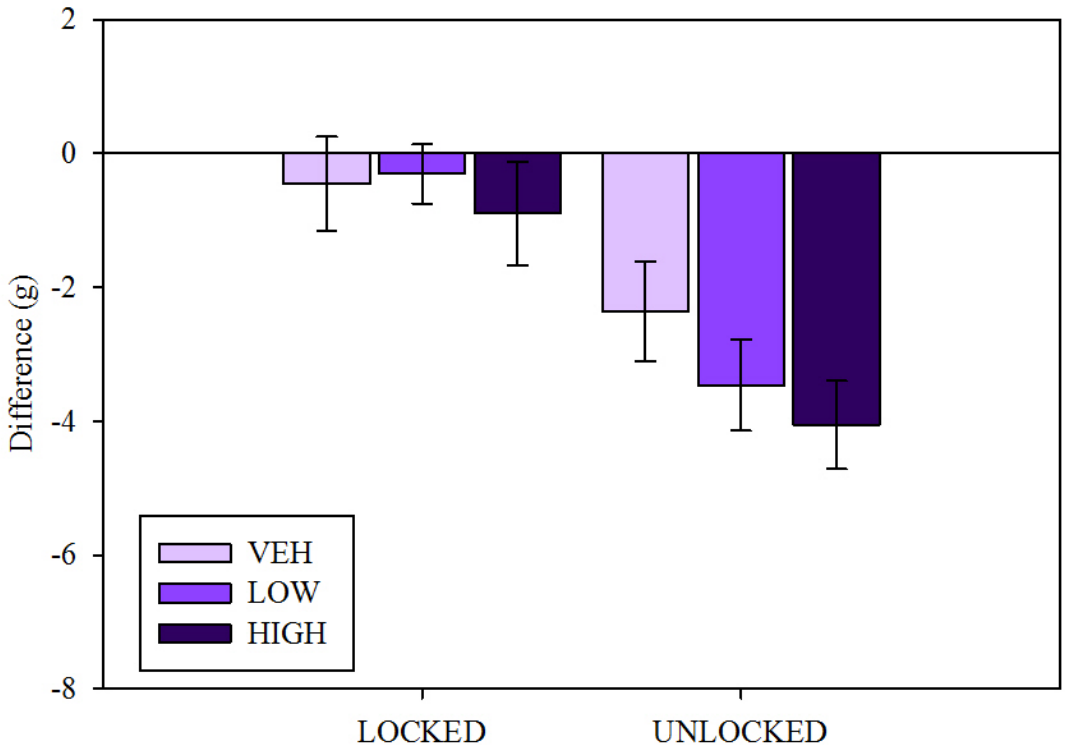

Figure 20. Mean (±SEM) feeding-suppression for rats in Experiment 4b. Data collected following 6 h periods of wheel-access (either 'Locked' or 'Unlocked') and an injection of the 0 ('VEH'), 0.125 ('LOW'), or 0.25 mg/kg ('HIGH') dose of THC. Differences are from baseline (2 days prior to wheel-access) food-consumption.