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EFFECTS OF CHLORPROMAZINE ON FEEDING AND WHEEL RUNNING IN RATS WITH ACUTE WHEEL ACCESS

By

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THESIS

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Abstract

Anorexia Nervosa (AN) is characterized by a self-imposed starvation and is often accompanied by excessive exercise that results in severe malnutrition and sometimes death. Behavioural and pharmacological treatments of AN need to be improved. In rats, acute 3 h daytime wheel access suppresses *ad lib* feeding over the subsequent night relative to no wheel controls, a phenomenon that has been suggested as an animal model of AN. This acute wheel induced feeding suppression (WIFS), can be induced reliably when rats are given limited wheel access exposure during the light cycle (Lattanzio & Eikelboom, 2003). The acute WIFS is useful because it can be used to test the effects of pharmacological agents, in a paradigm where the duration of wheel running and its effects are tightly defined. Chronic chlorpromazine injections can minimize the severity of activity anorexia (AA) procedure (Routtenberg, 1968), a related rat model of AN (Epling, Pierce & Stephan, 1983), by attenuating wheel running. Experiment 1 tested the acute effects of a one-time chlorpromazine injection (2 mg/kg IP) on the acute WIFS in 40 adult male rats. Animals were divided into five treatment groups (n=8): drug before wheel access (DW); drug after wheel access (WD); drug with locked wheel access (DNW); saline with locked wheel access (SNW); and saline with wheel access (SW). Half of each of the three control groups (DNW, SNW and SW) received injections before wheel access and half were injected after the wheel access period. Experiment 2 followed the same procedure except a broader range of chlorpromazine doses was tested (0.25, 0.50, 1 and 2 mg/kg). Both studies show that while chlorpromazine (at 1 and 2 mg/kg) did not attenuate feeding or wheel running, it blocked the acute WIFS. At doses of 0.25 and 0.50 mg/kg chlorpromazine had no effect.

Because the pharmacological profile of chlorpromazine implicates the serotonin, histamine and dopamine systems in the acute WIFS (and potentially AN), future work should look at drugs with more specific modes of action to identify which neurotransmitter systems may be involved. The acute WIFS procedure may be useful for screening potential drug treatments for AN, where exercise is often elevated and feeding is suppressed.

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Anorexia nervosa (AN) is a disorder that is characterized by excessive weight loss caused at least partially by reduced eating. Often, this condition is exacerbated by a constant general hyperactivity and compulsion to exercise (Attia & Walsh, 2009; Bergh, Erisson, Lindberg & Södersten, 1996; Casper, 2006). It has been observed that up to 85% of AN patients exercise to excessive levels throughout the course of the disorder (Davis, 1997) and some argue that the relationship between excessive activity and reduced feeding is a key feature of AN.

Current treatments for AN, most of which are behavioural in nature, are not very effective. Of all patients treated, only 46% fully recover, 34% improve and 20% develop a chronic form of the disease that, in many cases, leads to death (Steinhausen, 2002). Given the unfavourable prognosis of AN patents, it is evident that clinicians need to look beyond the traditional 'behavioural' treatment strategies and into other domains, such as the biochemical. To explore the biochemical substrates of human disease, researchers commonly turn to animal models.

With respect to AN, many wheel running animal models exist, all of which examine the relationship between feeding and exercise when it becomes counterintuitive. One possible advantage of such models is that they allow for the study of AN in the absence of the psychological and social factors, which may make our understanding of the disease difficult in humans. Once it is clear how feeding and exercise interact in AN, researchers will be in a position to study how psychological factors might contribute to the development and maintenance of this disease.

Wheel Running Models of Anorexia Nervosa

When a rat is given continuous access to a running wheel, it will run and gradually escalate its running from about 1 km on the first day up to 5-6 km over a period of a few weeks (Eikelboom & Mills, 1988). One interesting consequence of wheel introduction is a 25% self-imposed suppression in feeding lasting about 7 to 10 days and a chronic reduction in weight (Alfonso & Eikelboom, 2003). This has been labelled as the wheel induced feeding suppression (WIFS). This phenomenon is counterintuitive because the animals expend more calories running and consume less food than non-wheel controls. It has been proposed that this could function as a model of an important aspect of AN (Lattanzio & Eikelboom, 2003).

The animal model of AN that has attracted the most attention is the activity anorexia (AA) procedure (Casper, Sullivan & Tecott, 2008; Epling, Pierce & Stephan, 1983). This procedure is similar in some ways to the WIFS paradigm. In the AA procedure, wheel introduction and food restriction (usually 1 hour of food access a day) are introduced simultaneously and result in reduced feeding (relative to food restricted non-wheel controls) and increased running (relative to non-deprived controls) which proves fatal for rats within a few days (Routtenberg & Kuznesof, 1967). The interpretation of AA is complicated by a number of factors inherent to the procedure. Firstly, there is a learning complication in that animals must adapt to the experimenter-imposed feeding schedule. Thus, food intake generally increases over the first number of days and over cycles of this procedure in a manner that indicates learning (Boakes & Dywer, 1997; Hampstead et al., 2003; Lett et al., 2001; Paré et al., 1985). Secondly, with food deprivation, wheel running increases significantly (relative to *ad lib* fed rats) and thus energy expenditure is increased (Routtenberg & Kuznesof, 1967). This occurs even if the wheel is not novel (Exner et al., 2000; Nergårdh et al., 2007). Lastly, at wheel introduction feeding is

suppressed both with *ad lib* and restricted food access (Alfonso & Eikelboom, 2003; Routtenberg & Kuznesof, 1967). The feeding suppression induced by the wheel is temporary, both with *ad lib* feeding (Afonso & Eikelboom, 2003) and in the AA procedure (Hampstead et al., 2003). The problem in the activity anorexia procedure seems to be that animals do not have enough energy reserves to adapt to the various changes before the onset of starvation. It is not clear how or if these three factors are connected, or how they interact in the AA procedure, but they ultimately may all be important in AN. What is apparent is that the WIFS, because it does not involve food deprivation, provides a simpler model that may address specific aspects of the feeding-exercise relationship.

The WIFS seen with voluntary running and *ad lib* feeding, has many attributes that make it a useful animal model of the exercise feeding relationship in AN. Our model is considered to have obvious face validity for AN (Willner, 1990), as it reflects the negative relationship between exercise and feeding found in the human condition (Attia & Walsh, 2009; Bergh & Södersten, 1996; Casper, 2006). This isomorphic model of AN (Smith, 1989) reflects many aspects of the human disorder without specifying its etiology or mechanisms, which remain largely unknown but are easier to explore in the animal model. The most obvious benefit to the WIFS model is that all of the changes in feeding and running are intrinsically motivated, not externally imposed by an experimenter. The rat model shows a similar developmental profile to that seen in human AN. For example, prepubescent rats (less than 32 days old), even though they run as far as (or further than) their adult counterparts, do not show the typical feeding suppression observed when adolescent (36 – 41 days old) and adult (51+ days old) rats are given the opportunity to run (Dalton-Jez & Eikelboom, unpublished work). This highlights the potential for the WIFS as a model to study AN.

One very important characteristic of the WIFS model is that it can be elicited when rats are given short-term access to a wheel. It has been shown that 2 h of wheel access during the light cycle is enough to trigger this feeding suppression over the next night (Lattanzio & Eikelboom, 2003). One key benefit of testing the effects of limited daytime wheel access on feeding is that both the wheel and no wheel groups can feed without distraction during the dark cycle, when most eating occurs.

This acute WIFS may prove a valuable procedure for determining the neurochemical systems involved in this paradoxical exercise-feeding relationship. Using an acute WIFS model, a drug can be given to an animal before, after, or in the absence of, short term daytime wheel access, permitting an evaluation of the drug's effect on running, feeding, and the running-feeding interaction without the complication of constant wheel access. As drug tests in animal models have long been suggested as useful for preclinical drug evaluations (Mc Kinney, 1974), this procedure could ultimately lead to our understanding of the biochemical compounds and processes involved in AN and potential pharmaceutical treatments. The question that remains then, is what class of drugs should be the first explored in the acute wheel running model? Foundational research, conducted in the 1960s and 70s with the AA procedure, suggests that the antipsychotics might be a good place to start.

Experimental Foundations

The deadly relationship between *ad lib* wheel running and restricted (1 h per day) feeding in rats was first discovered in 1954 by Hall and Hanford. Soon after, researchers began to try and uncover the neural mechanisms behind AA. Initial work tested the effect of the antipsychotic chlorpromazine (CPZ) and the sedative pentobarbital (PTB), on the survival rates

of rats in the AA procedure. Routtenberg and Kuznesof (1967) injected rats daily with either CPZ (1 mg/kg), pentobarbital (4 mg/kg) or saline immediately after the 1 h feeding period in the AA procedure. Food consumption, body weight and wheel running were measured daily. Results indicated that pentobarbital was not helpful in improving survival. Rats injected with this agent ran excessively, did not adapt to food restriction, lost weight and died (as did non-injected controls). Chlorpromazine, on the other hand, was effective in significantly reducing wheel running and improving survival compared to saline injected control rats, allowing animals to stabilize body mass. Specifically, CPZ injected rats survived for the entire duration of the study and ran maximally 1500 wheel turns, while the saline injected controls reached exhaustion by day 8 and ran roughly 5000 wheel turns.

Although CPZ at 1 mg/kg improved survival, reducing wheel running compared to the saline group, not all rats injected were protected from starvation. In fact, 25% of them still perished. To determine the optimum dose of CPZ required to ensure survival of all AA rats, Routtenberg (1968) replicated his first experiment using a 2 mg/kg dose of CPZ. Rats were given daily IP injections over the course of the study. After 8 days of baseline, animals were put on a restricted (1h/day) diet and given *ad lib* access to either locked or unlocked wheels for 8 days. Again, wheel running, body weight and food consumption were measured. Data showed that 2 mg/kg injections of CPZ improved survival rates, and even more so than those injected with 1 mg/kg. Rats injected with 2 mg/kg ran significantly less (about 1000 revolutions by day 12) than those with only 1 mg/kg (roughly 3000 revolutions by day 12) and weighed about 25 g more. Interestingly though, the group injected with the higher dose of CPZ and with the higher survival rate, ate less than the 1 mg/kg group over the first 10 days of the experiment. One explanation for this might be that, at higher doses, CPZ produces a conditioned taste avoidance

(CTA), as described by Parker (2003), that enhances the feeding suppression observed when rats are introduced to the AA paradigm.

Taken together, these findings suggest that CPZ, a typical antipsychotic, can help animals cope in the AA paradigm by reducing activity. They also suggest that 2 mg/kg might be the optimum dose, as it seems to be the minimum required to ensure that 100% of the animals survive. These findings make it tempting to suggest CPZ as a potential treatment for AN, however it would be premature to do so. Although Routtenberg's studies answer many questions about the effect of CPZ on activity in the AA procedure, it is unclear how feeding is effected. This is especially true since the animals are externally food deprived, unlike in AN where the feeding suppression is self imposed. Furthermore, it is not clear why the CPZ injected rats ate less than the saline injected group. Was it because CPZ directly effected feeding? Or was it because CPZ impaired the animal's ability to learn? Unfortunately it is impossible to tease these two possibilities apart using the AA model alone. Moreover, as wheel access is continuous (except for feeding time) it is not clear when the drug should be administered to have the most effect, especially since CPZ's half life is known to range from 16 – 30 h when injected in humans (Yeung et al., 1993). In these early studies by Routtenberg's group, CPZ or saline was injected immediately after the 1 hour feeding period. Thus, administration was far removed from the next meal and could have resulted in learned taste avoidance (Parker, 2003). This could indirectly prevent a recovery of feeding, making it difficult to distinguish the avoidance from drug-induced reduction.

To address the limitations of the AA model and to answer the lingering questions from Routtenberg's important groundwork, it would be interesting to explore the effects of CPZ on the acute WIFS model. Using this paradigm the effect of CPZ on running, feeding and the running-

feeding interaction could be specifically identified. By testing similar doses to those used by Routtenberg we have a reference data set to which we can compare and contrast. This presents a great opportunity for us to build on historical work and take it to the next level. One point worth mentioning is the fact that during Routtenberg's time little was known about the effects of antipsychotics on feeding and weight gain. Today however, this is not the case and it may be useful to discuss the affects of antipsychotics, specifically chlorpromazine, on weight.

Chlorpromazine

Chlorpromazine (2-chloro-10-(3-dimethylaminopropyl-phenothiazine) represents one of the earliest known and clinically prescribed antipsychotics. In 1955 it was approved in the United States and the effect of this drug in emptying mental hospitals has been compared to that of penicillin on infectious disease (Turner, 2007).

CPZ affects a variety of receptors in the central nervous system, having antidopaminergic, anti antiserotonergic and antihistaminic properties which are discussed in detail below. Interestingly, CPZ tends to have a higher affinity at serotonin receptors than at dopamine receptors, which is the opposite effect of most other typical antipsychotics. Therefore, CPZ behaves more like an atypical antipsychotic in terms of its dopamine and serotonin activity (McKim, 2007). CPZ could help treat patients with AN by reducing the rewarding aspects of excessive exercise and starvation, through antagonizing the D1, D2, D3 and D4 receptors (Friedman, 1996), and by increasing appetite and feeding through blocking the HI and 5-HT2C receptors, respectively (Ravussin, Lillioja & Knowler, 1988; Gothelf, et al., 2002).

Antipsychotic Pharmacology: Antipsychotic action and Weight Gain

Antipsychotic drugs (also referred to as neuroleptics) are a group of psychoactive compounds that treat psychosis. Antipsychotics are lipophilic antagonists that effect behavior by binding to postsynaptic dopamine, serotonin and histamine receptors (amongst others) in different regions of the brain. Each antipsychotic has a slightly different pharmacologic profile but all block D2 receptors in the dopamine pathway causing dopamine to have less of an affect. Excess release of dopamine in the mesolimbic pathway has been correlated with psychotic disorders, such as schizophrenia, and these drugs are very effective at controlling symptoms (Friedman, 1996). In fact, an almost perfect correlation exists between the therapeutic dose of an antipsychotic and the drugs affinity for the D2 receptor. Therefore, a weak antagonist requires a larger dose to control psychotic symptoms and vice versa (Jones & Pillowsky, 2002).

The first class of antipsychotics (known as typical antipsychotics) involved the discovery of CPZ's affects in the 1950s. These early drugs, although effective in reducing psychotic symptoms, produce a number of serious side effects including weight gain. A second generation of drugs, called the atypical antipsychotics, has since been developed. Atypical antipsychotics, such as olanzapine, treat psychosis with fewer side effects but still lead to obesity (Allison et al., 1999).

Antipsychotic induced weight gain is caused by both a reduction in energy expenditure (Ravussin et al., 1988) and an increase in food intake (Gothelf et al., 2002). Support for this comes from reports that demonstrate lower than predicted resting energy expenditure in people taking antipsychotic drugs compared to age matched healthy and schizophrenic controls not taking the drug (Sharpe et al., 2005). A recent study demonstrated that patients taking clozapine (an atypical antipsychotic) burn 20% less energy and consume 20% more calories than the World

Health Organization's daily recommendations (Sharp et al., 2006), suggesting it as a possible treatment for AN.

Two receptors that are robustly associated with weight are the serotonin 2C (5-HT2C) and histamine 1 (H1) receptors. Chronic agonistic activation of the serotonin receptor decreases feeding and feeding behavior (De Vry & Schreiber, 2002), while blocking the receptor chronically induces food intake, despite satiety, leading to weight gain (Meguid et al.,2000). It is thought that antipsychotics lead to weight gain by chronically antagonizing the central 5-HT2C receptors which cause patients to overeat, regardless of the sensation of satiety (Reynolds, Hill & Kirk, 2006) Histamine receptors are also linked to appetite. Appetite is increased by the chronic antagonism of the central H1 receptors (Wirshing et al., 1999). Interestingly, the different receptor affinity of antipsychotics to 5-HT2C and H1 are correlated with their weight gain potential. For instance, clozapine and olanzapine (typical antipsychotics), which have the highest affinity for 5-HT2C and H1 induce the greatest weight gain. In contrast, Risperidone (another typical antipsychotic) has a lower affinity for the 5-HT2C and H1 receptors, causing lesser weight gain (Virk et al., 2004). Although the 5-HT2C and H1 receptors are the most robust biological factors contributing to weight gain, the role of other neuroendrocrine factors such as ghrelin, leptin, orexin and prolactin is unclear (Rege, 2008). Further research is required to uncover their mechanisms and thus we will leave them out of further discussion.

To date, there is an enormous body of research identifying the medical properties of antipsychotics and their effects on brain chemistry and behavior (see Parraga, 2007 for a comprehensive example) but it is unclear how these drugs effect energy balance in patients with AN. To gain insight into this question (and to better understand the neural substrates of the wheel running rat model) the effects of CPZ on feeding, exercise and the interaction of these two

variables in the acute WIFS paradigm will be investigated.

The Current Study

It has long been evident that antipsychotic drugs commonly induce weight gain as a side effect (Allison et al., 1999). There has been speculation as to whether these drugs could help in the initial phases of AN treatment, because of their weight gain inducing qualities and the possible alleviation of associated psychological symptoms (fear of fatness, misperception of body mass). Recently, a randomized, double blind, placebo controlled trial of olanzapine, an atypical antipsychotic, was conducted using female AN patients as participants (Bissada et al., 2008). Olanzapine use resulted in increased weight gain and lessening of obsessive symptoms and has been suggested as a valuable option for the initial, short term phase of treatment. In light of these results, it would be prudent to test antipsychotics in the acute WIFS procedure.

In the current study CPZ, the typical antipsychotic, was evaluated using the acute WIFS procedure. This was the first of many drugs tested in the activity anorexia procedure (Routtenberg, 1968; Routtenberg & Kunzesof, 1967; Woods & Routtenberg, 1971). Chronic administration of CPZ in the activity anorexia paradigm reduces wheel running and so indirectly decreases the severity of the procedure. While these studies do provide a model in which to test drugs, the model is limited by the aforementioned complications of the AA procedure. In the current study, the acute WIFS model is used to evaluate the impact of acute CPZ on feeding, running, and the WIFS. Based on chronic work, it may be that CPZ (and similar drugs) directly stimulates or suppresses feeding which would then be evident in non-wheel controls. Alternatively, it may directly reduce activity and thus indirectly prevent the WIFS, tested by comparing groups given the drug before or after the limited wheel exposure. A third more

interesting possibility is that the exercise-feeding suppression dyad may be prevented by this drug. Such a drug would then target the relationship between running and feeding without increasing eating in non-exercising control animals or decreasing exercise, specifically removing the threat of this harmful relationship. If this dyad is an important aspect of the etiology of AN, it would suggest this class of drugs might prove useful in treatment of this puzzling disorder.

Experiment 1: As published by Adams, Parfeniuk & Eikelboom, 2009

Method

Subjects

Fourty male Sprague-Dawley rats (Charles River Canada, St. Constant, Quebec, Canada) weighing 200-225 g (47-49 days old) upon arrival were housed individually in standard shoebox cages (20 x 24 x 45 cm) and maintained on a 12 hour light/dark cycle, with lights on at 0700 (7:00 AM). Colony conditions were kept stable (50% relative humidity, 21-22 °C), and food and tap water were available *ad libitum* throughout the study. All experimental procedures were approved by the Wilfrid Laurier University Animal Care Committee which follows the policies and guidelines of the Canadian Council on Animal Care.

Apparatus

Wheel access was given in Nalgene™ running wheels (33 cm diameter and 11 cm wide) inserted in standard shoebox cages. These wheels could be locked using two paper clamps clipped between the rungs on the outside of the Nalgene wheel to prevent wheel turning. Wheel turns were counted to a resolution of 1 s using a magnetic closure system and the VitalView™ Minimitter Co. Ltd. software package.

Drug and doses

Chlorpromazine solution (CPZ, as chlorpromazine hydroxide, Sigma Aldrich, St. Louis, MO) was prepared fresh on each injection day in sterile isotonic saline at a dose of 2.0 mg/kg. CPZ was injected intraperitoneally (IP) at a volume of 1 ml/kg. The control rats that did not receive CPZ received equivalent injections of saline.

Procedure

Baseline measures of food consumption, water consumption and weight were taken daily at about 1430 (2:30 PM). Food consumption was measured by dumping the food pellets from the lid of each cage into separate plastic dishes and weighing them on a digital scale that was accurate to one tenth of a gram. Daily food consumption was determined by calculating the differences in food weight from one day to the next. Small crumbs and food particles were ignored, as in previous work they have been found to weigh less than 1 g, and not to vary across conditions. Rats initially received 300 g of food at the start of the experiment and were topped up to this amount once their total available food volume fell below 200 g. Water bottles were weighed daily, also at around 1430, and the difference from one day to the next indicated how many ml each rat consumed. Once a bottle fell below half of its capacity (which was about 600 ml) it was topped up. Body weight was measured daily between 1430 (2:30 PM) and 1530 (3:30 PM). After 7 days of baseline, all 40 rats were moved into wheel cages for 24 h to establish a baseline running level, measure the WIFS seen with one day of *ad lib* wheel access and provide rats familiarity with the wheel. The rats were then assigned in a way to equalize for feeding suppression (day before wheel running – the day after wheel running), distance run (wheel turns) and body weight (g) into 5 groups of 8. Twenty-four h after the wheel exposure period rats were rank ordered from smallest to largest in terms of their WIFS and then distributed to 1 of the 5

treatment groups in the following manner: Rat 1 into group 1, Rat 2 into group 2, Rat 3 into group 3, Rat 4 into group 4, Rat 5 into group 5, Rat 6 into group 1, Rat 7 into group 2, Rat 8 into group 3 etc. After all the rats had been distributed in terms of their WIFS an analysis of variance (ANOVA) was completed to ensure that none of the groups differed in terms of their WIFS, wheel turns or body weight. If any groups differed at this point the animals were shifted around using feeding and weight data until all groups were equal.

The five groups were the drug before wheel access (DW); drug after wheel access (WD); drug with locked wheel access (DNW); saline with locked wheel access (SNW); and saline with wheel access (SW). Half of each of the three control groups (DNW, SNW and SW) received their injections before wheel access and half were injected after the wheel access period (a 3 group by 2 injection time ANOVA revealed no significant differences in feeding on the critical day due to injection time, so the two sub-groups for each group were combined for the final analysis).

Three days after the 24 h baseline wheel exposure, animals injected before wheel access received the appropriate IP injections, either saline or CPZ, at approximately 1500 and were placed back into their home cages. Thirty minutes after their injection (about 1530), all rats were placed in the wheel cages, with the wheel unlocked for wheel access or locked for novel environment only groups, and remained there for 3 h (during which food was not accessible). The rats were then placed back in their home cages 30 min before the lights went out. Groups that received their injection after wheel access were injected immediately after being removed from the wheel cage. Each rat received only one injection. Food consumption over the next 22 hours was measured as the critical dependent variable.

Results

Initial 24 h baseline access

Figure 1 shows wheel turn data over the 24 h baseline running period. Because of the way the animals were assigned a 5 group ANOVA revealed that the experimental groups did not differ significantly. Wheel turns in this period did not correlate with later 3 h running, or with the decrease in feeding induced by the 24 h of this baseline wheel access.

Figure 2 shows food consumption for the day before and the day after wheel access (24 h). Data was analyzed using a 5 group by 2 day mixed ANOVA which revealed an overall significant feeding suppression [F (1,35) = 52.26, $p < .001$], but again, because rats were assigned to groups based on their feeding suppression, the groups did not differ. Rats ate 29.8 ± .52 (SEM) g the day before the 24 h wheel access and 25.0 ± 0.58 g over the 24 h of wheel access.

Running on injection days (3 h wheel access).

A 3 group ANOVA revealed that the wheel groups (DW, WD, SW) did not differ significantly in their running over the three hours of wheel access, as measured in wheel turns (see Figure 3).

Feeding after injection and 3 h wheel access.

Figure 4 depicts feeding over the 24 h after injection of CPZ or saline before or after 3 h access to a locked or unlocked wheel. Overall, the 5 group ANOVA revealed the food consumption of the groups differed significantly [F (4,35) = 3.82, $p < 0.05$]. A Newman-Keuls post hoc test found that only the saline injected, wheel exposed rats (Group SW) ate less than the rats in the other four groups, $p < 0.05$.

In the three wheel groups, feeding suppressions (difference between the 24 h before wheel access and the 24 h afterwards) differed [$F(2,21) = 18.49$, $p < 0.001$]. Again, post hoc tests show that the SW group was responsible for this effect, being the only wheel group that showed a feeding suppression (data not depicted).

There was also no correlation between number of wheel turns and feeding in the 24 h after 3 h wheel exposure (for the 24 rats with wheel access the Pearson r = -0.039).

Discussion

Experiment 1 suggests that 2 mg/kg of CPZ specifically prevents the interaction of running and feeding in the acute WIFS paradigm. This is true since CPZ did not elevate feeding in the DNW group relative to the SNW group or reduce running in the DW group relative to the WD group but did block the running-feeding interaction (in the DW and WD groups compared to the SW group). These results are very interesting and suggest CPZ may be useful in identifying the neural substrates of the WIFS (and potentially AN). Before we can begin to understand how CPZ may act as a possible treatment for the acute WIFS paradigm and thus AN, more questions need to be resolved: First, are these results repeatable? Second, what minimum dose is required to observe this effect? And finally, is this a dose dependent or an "all or none" effect? Experiment 2 was designed to answer these questions.

Experiment 2

Methods

Subjects

Sixty-four male Sprague-Dawley rats (Charles River Canada, St. Constant, Quebec, Canada) were housed in standard shoebox cages (20 x 24 x 45 cm) and kept on a 12 h light-dark cycle, with lights on at 1200 (noon). Colony conditions were kept stable (50% relative humidity, 21 – 22°C), and food and tap water were available *ad libitum* throughout. The animals had a history of saccharin consumption from a previous experiment and were therefore given 10 days to acclimatize to their "non-saccharin" diets before baseline food and water measures commenced. The animals weighed between 450 – 550 g at the start of the experiment and all treatment groups were counterbalanced with respect to saccharin history. All experimental procedures were approved by the Wilfrid Laurier University Animal Care Committee which follows the policies and guidelines of the Canadian Council on Animal Care.

Apparatus

The equipment used here were the same as in Experiment 1.

Drug and doses

Chlorpromazine solution was prepared fresh on each injection day in sterile isotonic saline at doses of 2.00, 1.00, 0.50 and 0.25 mg/kg. CPZ was injected intraperitoneally (IP) at a volume of 1 ml/kg. The control rats that did not receive CPZ received equivalent injections of saline.

Procedure

Baseline measures of food consumption, water consumption and weight were taken daily

at about 1500. Food, water and wheel turn data were collected using the same procedures as in Experiment 1. Due to equipment limitations, Experiment 2 was conducted in two equivalent replications of 32 rats. After collecting 7 days of baseline data (food consumption [g] and body weight [g]), rats were given 24 h of wheel access to establish baseline levels of running, measure the WIFS seen with one day of *ad lib* wheel access and provide rats with familiarity to the wheel. The rats were then randomly assigned to equalize wheel running and feeding suppression to 8 groups of 8: 2 mg/kg drug before wheel access (2-DW); 2 mg/kg drug after wheel access (2-WD); 1 mg/kg drug before wheel access (1-DW); 0.5 mg/kg drug before wheel access (.5-DW); 0.25 mg/kg before wheel access (.25-DW); 2 mg/kg drug with locked wheel access (2-DNW); saline with locked wheel access (SNW); and saline with wheel access (SW).

Three days after the 24 h baseline wheel exposure, animals injected before wheel access received the appropriate injections, either saline or CPZ, at approximately 1800, 4 h before lights out. Thirty minutes after their injection (about 1830), all rats were placed in wheel cages, with the wheel unlocked for wheel access or locked for novel environment only groups, and remained there for 3 h. The rats were then placed back in their home cages. Groups that received their injection after wheel access were injected immediately after being removed from the wheel cage. Each rat received only one injection. Food consumption over the next 24 h was measured as the critical dependent variable.

Results

Initial 24 h baseline access

Since animals were assigned to groups to equalize distance run, an 8 group analysis of variance (ANOVA) revealed that the groups did not differ significantly in wheel turns over the baseline day (see Figure 5). Similar to Experiment 1, the distance run over the initial 24 h wheel access did not correlate with later 3 h running, or with the decrease in feeding induced by the 24 h wheel access.

An 8 group by 2 day mixed ANOVA looking at food consumption (g) on the test day, 24 h after the 3 h wheel exposure, revealed an overall significant effect on feeding [$F(1,56) = 552.9$, $p < 0.001$] and that none of the treatment groups differed. This indicates that the experimental groups were successfully balanced for their level of feeding suppression. Rats consumed 36.8 ± 0.52 (SEM) g the day before wheel access and 31.0 ± .58 g over the 24 h of wheel access (see Figure 6).

Running on injection days (3 h access).

The 5 group ANOVA revealed that the 5 wheel groups (.25-DW, .5-DW, 1-DW, 2-DW, 2-WD) did not differ significantly in their running over the three hours of wheel access, as measured in wheel turns (see Figure 7). There was also no significant correlation between the number of wheel turns and feeding in the 24 h after 3 h wheel exposure (for the 40 rats with wheel access the Pearson r = -.167).

Feeding after injection and 3 h wheel access.

Figure 8 shows feeding over the 24 h after injection of CPZ (at 2.00, 1.00, 0.50 or 0.25 mg/kg) or saline before or after 3 h access to a locked or unlocked wheel. Overall, the 8 group ANOVA revealed a significant main effect on feeding [F (7,55) = 5.292, $p < .001$]. A Newman-Keuls post hoc test revealed that the SW group ate less than the SNW, 1-DW, 2-DW, 2-WD and 2-DNW groups and that the .25-DW and .5-DW groups ate an intermediate amount of food compared to the SW and 2-DW/2-WD groups.

Discussion

Two mg/kg of CPZ prevented the acute WIFS, replicating the effects observed in Experiment 1. It was also determined that a dose of 1 mg/kg may be the minimum required to prevent a feeding change when rats gain access to the wheel. Furthermore, Experiment 2 revealed that the effects of CPZ on the acute WIFS are dose dependent (and not "all or none").

General Discussion

The Present Study

Using the WIFS, a simple model that focuses on a specific aspect of the AA procedure, we are able to dissect some of the links between exercise and feeding. In particular this acute WIFS procedure can be used as a simple screen to assess the acute behavioural effects of drugs, in this case CPZ, and elucidate how the drug works to impact feeding in the WIFS. In this procedure, the direct effect of the drug on feeding is evident in the comparison between the saline and drug groups that did not have wheel access. In the present study it was shown that acute injections of CPZ have do not directly effect feeding. The comparison between the two saline groups with and with out wheel access demonstrates that the procedure supports a feeding

suppression. Further comparison of rats receiving CPZ before or after wheel access demonstrated that drug had no effect on acute daytime wheel running. Finally the comparison of the CPZ wheel groups with the CPZ no wheel group demonstrated that the drug prevents the acute WIFS.

Chlorpromazine's effect on wheel running in the Acute WIFS

Based on Routtenberg's chronic work (1967), it may be expected that the DW groups would have run less than the SW and WD groups, but this was not the case. In fact, all wheel groups, regardless of their injection history, ran equivalent distances. Reasons for this are not totally clear. Simply stated, it might be that CPZ, when injected acutely, does not affect wheel running. A second possibility however, could be that the acute WIFS model, which permits only 3 h of daytime wheel access, is not sensitive enough to detect running differences when CPZ is injected between 0 and 2 mg/kg. Perhaps in future studies, daytime wheel access duration should be extended. This might allow for running differences to be revealed (or completely ruled out), while preserving the model's key strength - which is to determine the effect of drugs on running, feeding and the running feeding interaction.

In the acute WIFS model, it is interesting how little daytime running, around 400 wheel turns, is necessary to suppress feeding over the subsequent night. This is consistent with previous work that 2 h of daytime wheel access can suppress feeding as much as *ad lib* wheel access (Lattanzio & Eikelboom, 2003) and that no correlation exists between running and the feeding suppression (Afonso & Eikelboom, 2003). To date, most animal studies of AN have focused on the effects of food restriction on wheel running in the AA model, which becomes excessive. The current model however looks directly at the opposite; the effects of limited wheel

access on feeding. In this case, excessive wheel running is not critical to the model, a phenomenon that enables us to test the effects of drugs specifically on the running feeding interaction.

Chlorpromazine's effect of feeding in the acute WIFS

In the present experiments, animals that received a saline or a low dose CPZ injection (less than 1 mg/kg), either before or after wheel access, were the only animals that showed the anticipated feeding suppression. At doses equal to or greater than 1 mg/kg, CPZ prevented the feeding suppression when injected either before or after wheel exposure. That the non-wheel CPZ injected controls did not eat significantly more than the saline control animals indicates that the drug, when injected acutely, does not increase appetite or food intake overall. This is contrary to what is observed when CPZ is administered chronically (Allison et al., 1999). Reasons for this are unclear. It might be that the effects of CPZ on the serotonin and histamine systems (and thus feeding and weight gain) change with repeated exposure. To understand what is happening, researchers will have to identify the physiological changes that accompany chronic CPZ exposure. Do the feeding centers in the brain become more sensitive to the antagonizing effects of CPZ? Are specific receptors up or down regulated? Answers to these questions may be important in helping us understand feeding, and pathologies of feeding.

In the current experiment running did not differ significantly between the groups so it appears this acute CPZ administration did not decrease activity and in this way circumvent the WIFS phenomenon. Results from Experiment 2, our dose-response analysis of CPZ effects on the acute WIFS, suggest that there may be an 'optimal dose range' that does not significantly reduce running with acute application, but does prevent the WIFS. Although we cannot confirm

the upper end of this 'optimal dose range', it appears that 1 mg/kg may be the minimum required. 2 mg/kg was chosen to mirror previous work with CPZ in AA, in which 2 mg/kg were used (Routtenberg, 1968; Routtenberg & Kuzeneof, 1967; Woods & Routtenberg, 1971). It appears that CPZ targets the relationship between feeding and activity in the running wheel, specifically uncoupling them to avoid the counterintuitive reduction in feeding that often accompanies running. The exact biochemical mechanisms underlying these results are not yet clear but hypotheses can be made.

Why might Chlorpromazine prevent the running feeding interaction?

Currently the biological underpinnings of the WIFS are unknown. Based on the present results however, combined with related literature, a working hypothesis can be deduced. Bouts of exercise, both voluntary wheel running (Lattanzio & Eikelboom, 2003) and forced treadmill exercise (Oscal, 1973), are known to increase the activity of the central serotonin (Jacobs, 1994; Chaouloff, 1997), histamine (Ransford, 1982) and dopamine (Dunn et al., 1996) systems in rats. Following wheel introduction, rats show a temporary feeding suppression compared to non-running controls, a finding that has been consistently reported in AA (Lett & Grant, 2001), WIFS (Lattanzio & Eikelboom, 2003) and acute WIFS (Adams & Eikelboom, unpublished work) procedures. From these results it can be proposed that running and feeding may interact via the serotonin and histamine systems. To investigate this further, more specific agonists and antagonists need to be tested. Running may cause a feeding suppression through the release of serotonin and histamine, which in turn act on the 5-HT2C and H1 receptors, respectively, to reduce appetite and food intake. This is supported by the widely accepted notion that 5-HT and histamine pathways play a fundamental role in energy balance (Vickers et al., 2000). Dopamine

is also released during wheel running (Dunn et al., 1996) and may play a significant role in reinforcing these maladaptive behaviours, encouraging them to potentially propagate to dangerous levels, like in the model AA or AN itself.

If this theory is accurate it may explain how CPZ acts to specifically prevent the feeding suppression in the acute WIFS model. At doses of 1-2 mg/kg, CPZ potentially antagonizes the 5HT2C and HI receptors, enough to counteract the suppressive effects of running on appetite and feeding, but not enough to increase feeding in the drug injected non-running control rats. The antidopaminergic properties of CPZ might also have helped by reducing the rewarding aspects of engaging in these counterintuitive behaviours. This framework then, highlights at least three systems that may potentially be involved in the WIFS, AA and AN.

To identify more specifically the potential roles of the serotonin, histamine and dopamine systems in the WIFS, future work could test drugs that act specifically on a single receptor type in the acute WIFS paradigm. For example, it would be interesting to independently test the effects of the specific 5-HT2C serotonin antagonist SB-242084 (Kennett et al., 1997), the dopamine D2 receptor blocker pimozide (Lambert & Porter, 1992), and the H1 receptor antagonist doxepin (Kanba & Richelson, 1983) to see how each of these would effect running and feeding in the acute WIFS model. Results from such studies would indicate which receptor, or combination of receptors, is important in preventing the interaction between running and feeding in rats. This in turn could help uncover the neurochemical substrates of the WIFS and potentially AN.

It should be noted that the above explanation presents a simplified view of the complex biochemical reactions that likely take place in the WIFS, AA and AN. The purpose of this discussion is to provide a foundation on which future hypotheses can be tested. There are likely

many factors and neurochemical systems contributing to the counterintuitive relationship that sometimes exists between feeding and exercise, only a few of which are touched on here.

Future Avenues of Exploration

To date, most clinical therapies of AN involve the administration of selective serotonin reuptake inhibitors, which are effective in treating the psychopathology but are ineffective in producing weight gain (Bergh et al., 1996). These results are puzzling, especially since 5-HT2C receptor antagonists are known to induce weight gain in non-anorexic humans (Meguid, Fetissov & Verma, 2000), and suggest that serotonin may not play a major role in linking exercise to feeding, at least in underweight AN patients. It would be interesting to test the effects of specific serotonin reuptake inhibitors on feeding and wheel running in the acute WIFS model. Results from such studies would help increase the models validity if they paralleled those observed in a human clinical setting.

A more interesting direction however, might be to test the effects of specific H1 antagonists on the acute WIFS. Results from this work may implicate the central histamine system as being the major biological link between feeding and exercise in wheel running rats, and potentially AN. The central histamine system has long been implicated in feeding. Central histaminergic activity is increased by food intake after starvation (Itoh, Oishi & Saeki, 1991) and H1 receptor concentration is correlated with food intake, particularly in low protein diets (Mercer, et al. 1994). Interestingly, AN patients have recently been found to have higher central H1 receptor densities compared to that of healthy controls participants (Yoshizawa et al., 2009). Identifying the effects of specific H1 antagonists on rats in the acute WIFS model could help validate the model and lead researchers to explore this exciting avenue.

The current experiment using the WIFS model involved acute, one time exposure to the drug, prior to application of an AN model. While the acute condition is valuable for screening this and other drugs for effectiveness, in reality, these drugs would be given chronically to humans. Thus, further study involving chronic access to CPZ and repeated access to the wheel is needed.

Prior work has suggested that 2 h a day of daytime wheel access produces a feeding suppression that lasts for 7 to 10 days (Lattanzio & Eikelboom, 2003), so this experimental protocol can be used to test drugs in a more chronic application. Also, rats could be injected with CPZ (or the drug of interest) several days before wheel access, a strategy that has been used effectively in the past (Routtenberg, 1968). Based on the early work (Routtenberg, 1968; Routtenberg & Kuzeneof, 1967; Woods & Routtenberg, 1971), it might be expected that with a higher CPZ dose or chronic administration of the drug, that the drug before and drug after wheel groups might differ in wheel running.

Conclusions

The present study suggests that injections of CPZ can eliminate the negative interaction between feeding and exercise observed in the acute WIFS preparation. Although these finding may prove helpful in identifying the neurochemical substrates of the WIFS and ultimately AN, they may raise more questions than answers. For example, although we have discovered that CPZ prevents the WIFS, the specific neurochemical actions of the drug that cause this effect can only be speculated upon. Here we have described the potential role of the serotonin, histamine and dopamine systems, the first two of which are linked to feeding and the latter to motivation and reward. Currently, it is not clear if it is CPZ's effect on one, two or all three of these systems

that is important in preventing the WIFS. To understand this, multiple studies would need to be conducted testing a battery of drugs that bind to a specific complement of these receptors. This would allow researchers to determine which receptors and brain regions are important in preventing the negative interaction between feeding and running observed in wheel running rats (and potentially in AN). Furthermore, once a drug has been identified that specifically acts on the receptors sites involved in the WIFS, researchers will have to investigate the effects of chronically administering this drug. Also, as AN is predominantly observed in women, our results need to be replicated in female rats.

The current experiments suggest that the acute WIFS paradigm may act as a legitimate "drug screening" model for AN but there are many challenges ahead. To develop this model further would require a massive time commitment and money investment. Although the resources to continue this work are not currently available, the goal of this project was to identify the potential for an acute "pre-clinical" rat model of AN to give us insight on how to treat patients more effectively.

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Figure Captions

Figure 1. Mean (±SEM) wheel turns over the initial 24 h wheel exposure. Animals were assigned to the chlorpromazine-wheel (DW), wheel-chlorpromazine (WD), saline-wheel (SW), chlorpromazine-locked wheel (DNW) and saline-locked wheel (SNW) groups to balance for distance run.

Figure 2. Mean (±SEM) food consumption (g) over the 24 h before (PreWheel) and after (PostWheel) the initial 24 h wheel exposure. Rats were assigned to the chlorpromazine-wheel (DW), wheel-chlorpromazine (WD), saline-wheel (SW), chlorpromazine-locked wheel (DNW) and saline-locked wheel (SNW) groups based on their level of feeding suppression.

Figure 3. Mean (±SEM) wheel turns (3 h access, paired with an injection). No significant differences existed between any of the chlorpromazine-wheel (DW), wheel-chlorpromazine (WD), saline-wheel (SW).

Figure 4. Mean (±SEM) food consumption (g) in the 24 h following chlorpromazine (D) or saline (S) injection and 3 h of wheel (W) or locked wheel (NW) access. Group DW was injected before, whereas group WD was injected after wheel access. *Significantly different from other four groups (NewmanKeuls $p<.05$).

Figure 5. Mean (±SEM) wheel turns over the initial 24h wheel exposure. The 2 mg/kg chlorpromazine-wheel (2-DW), wheel-2 mg/kg chlorpromazine (2-WD), 1 mg/kg chlorpromazine-wheel (1-DW), .50 mg/kg chlorpromazine-wheel (.5-DW), .25 mg/kg chlorpromazine-wheel (.25-DW), saline-wheel (SW), saline-locked wheel (SNW) and 2 mg/kg chlorpromazine-locked wheel (2-DNW) groups were balanced for distance run.

Figure 6. Mean (±SEM) food consumption (g) before (PreWheel) and after (PostWheel) the initial 24 h wheel access. The 2 mg/kg chlorpromazine-wheel (2-DW), wheel-2 mg/kg chlorpromazine (2-WD), 1 mg/kg chlorpromazine-wheel (1-DW), .50 mg/kg chlorpromazine-

wheel (.5-DW), .25 mg/kg chlorpromazine-wheel (.25-DW), saline-wheel (SW), saline-locked wheel (SNW) and 2 mg/kg chlorpromazine-locked wheel (2-DNW) groups were balanced for their level of feeding suppression.

Figure 7. Mean (±SEM) wheel turns (3 h access paired with an injection) No significant differences between the 2 mg/kg chlorpromazine-wheel (2-DW), wheel-2 mg/kg chlorpromazine (2-WD), 1 mg/kg chlorpromazine-wheel (1-DW), .50 mg/kg chlorpromazine-wheel (.5-DW), .25 mg/kg chlorpromazine-wheel (.25-DW), saline-wheel (SW).

Figure 8. Mean (SEM) food consumption (g) in the 24 h following chlorpromazine (D) or saline (S) injection and 3 h of wheel (W) or locked wheel (NW) access. Group DW was injected before, whereas group WD was injected after wheel access. The SW group consumed *significantly less then all groups except the 5-WD and .25-WD (which consumed intermediate amounts, statistically equal to all other groups) as per the NewmanKeuls post hoc, $p<.05$.

Figure 1

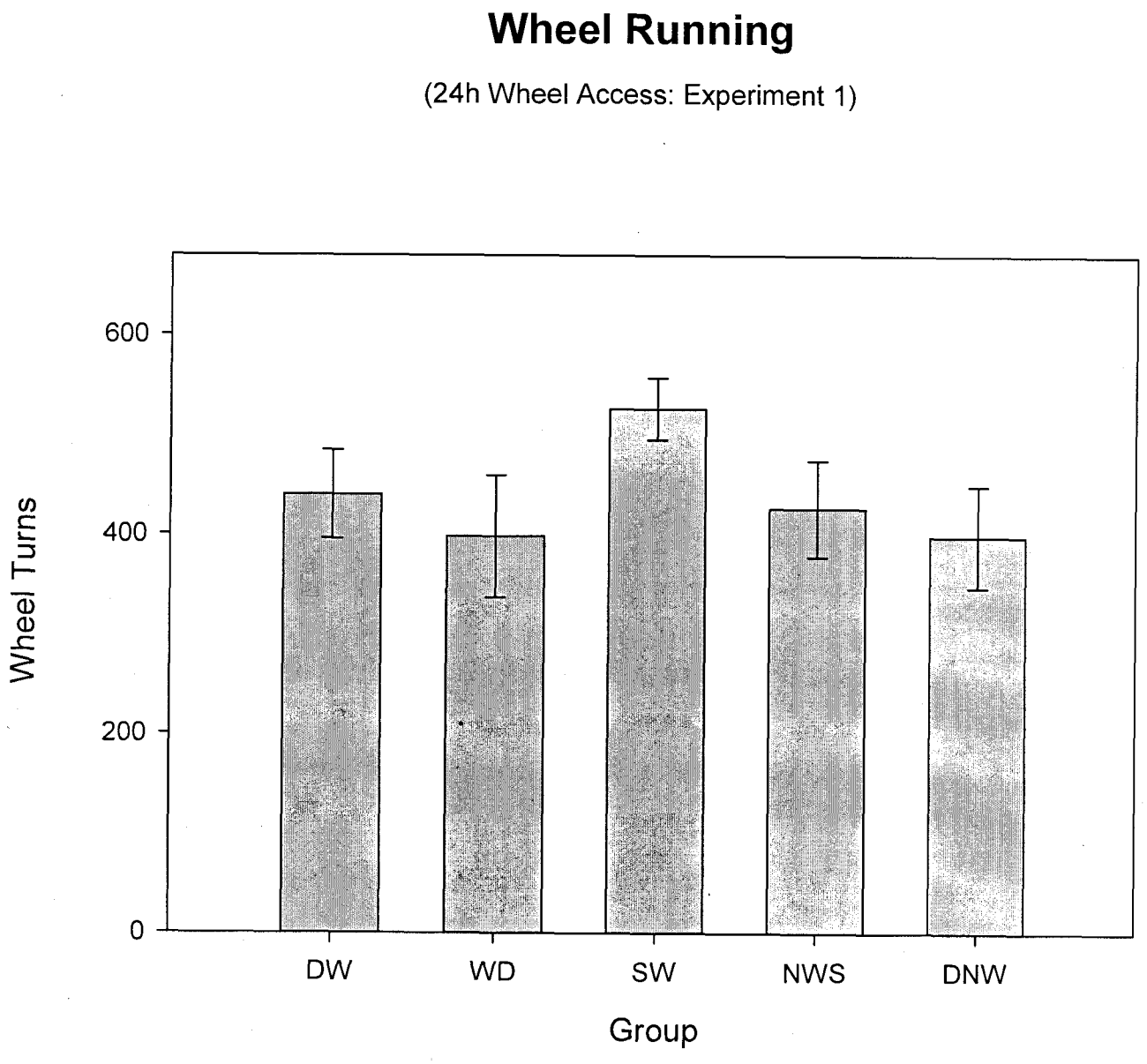

Wheel Running
(24h Wheel Access: Experiment 1)
Wheel Turns
600
400
200
0
DW
WD
SW
NWS
DNW
Group

Figure 2

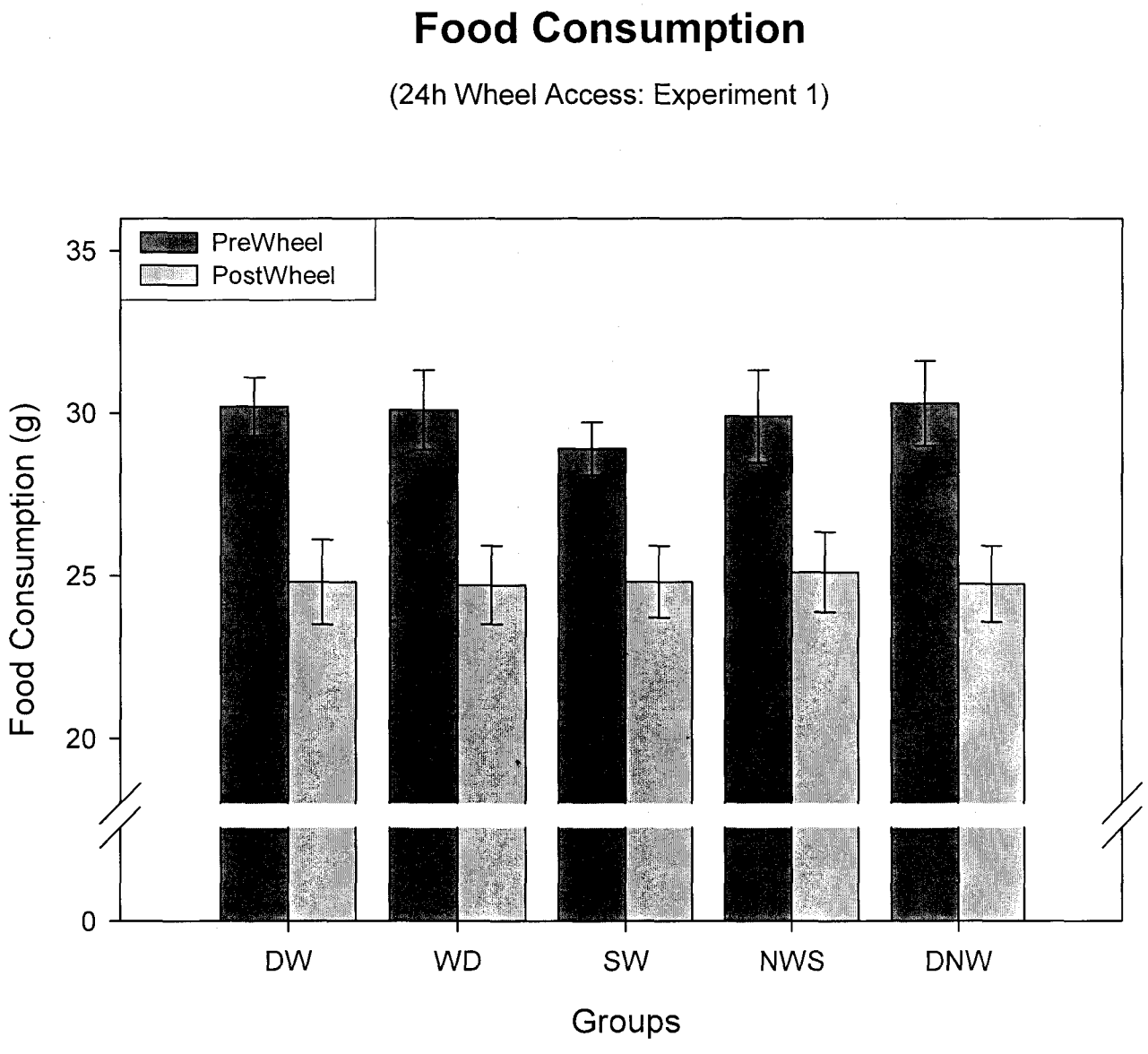

Food Consumption
(24h Wheel Access: Experiment 1)
PreWheel
PostWheel
Food Consumption (g)
35
30
25
20
0
DW
WD
SW
NWS
DNW
Groups

Figure 3

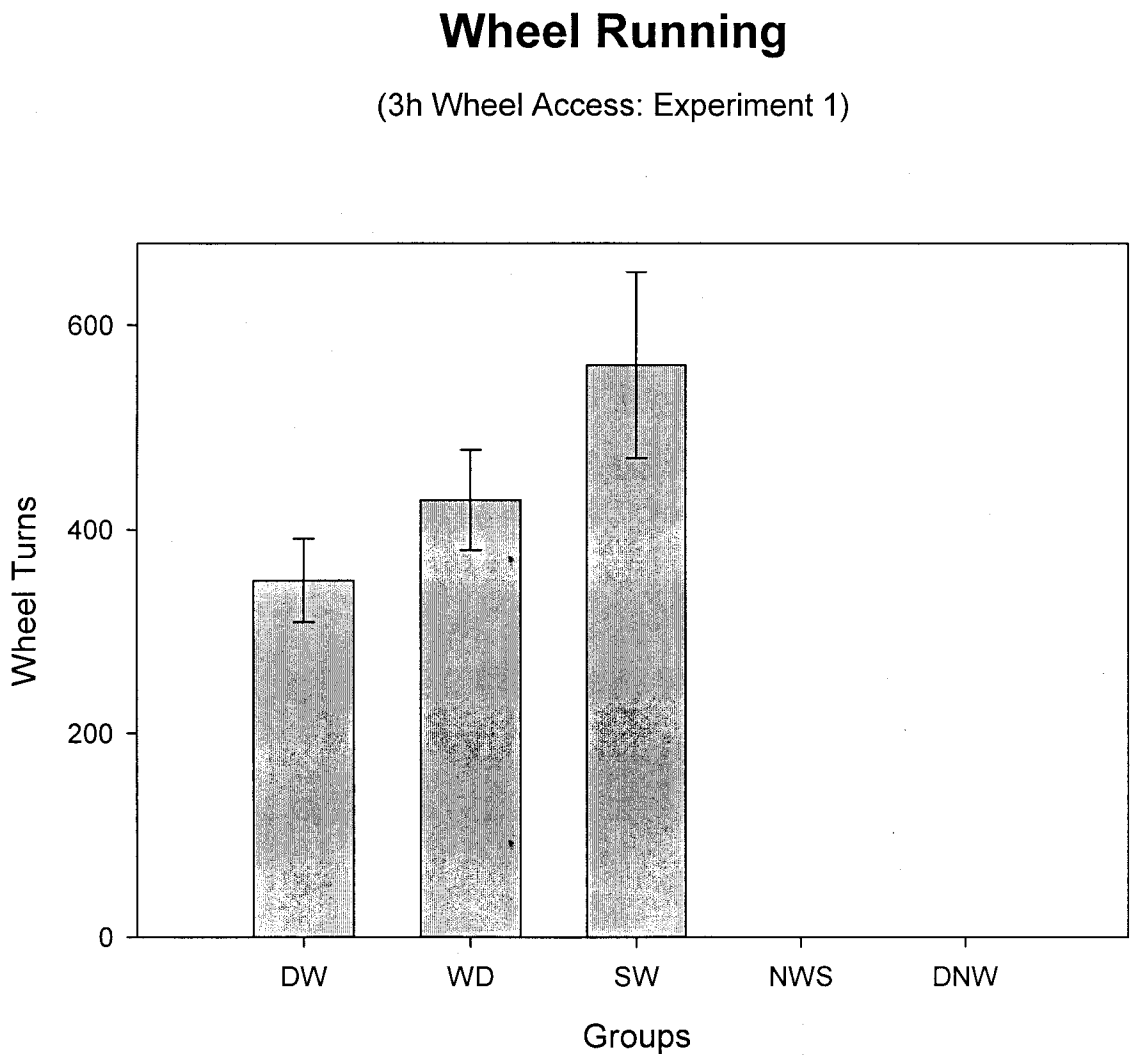

Wheel Running
(3h Wheel Access: Experiment 1)
Wheel Turns
600
400
200
0
DW
WD
SW
NWS
DNW
Groups

Figure 4

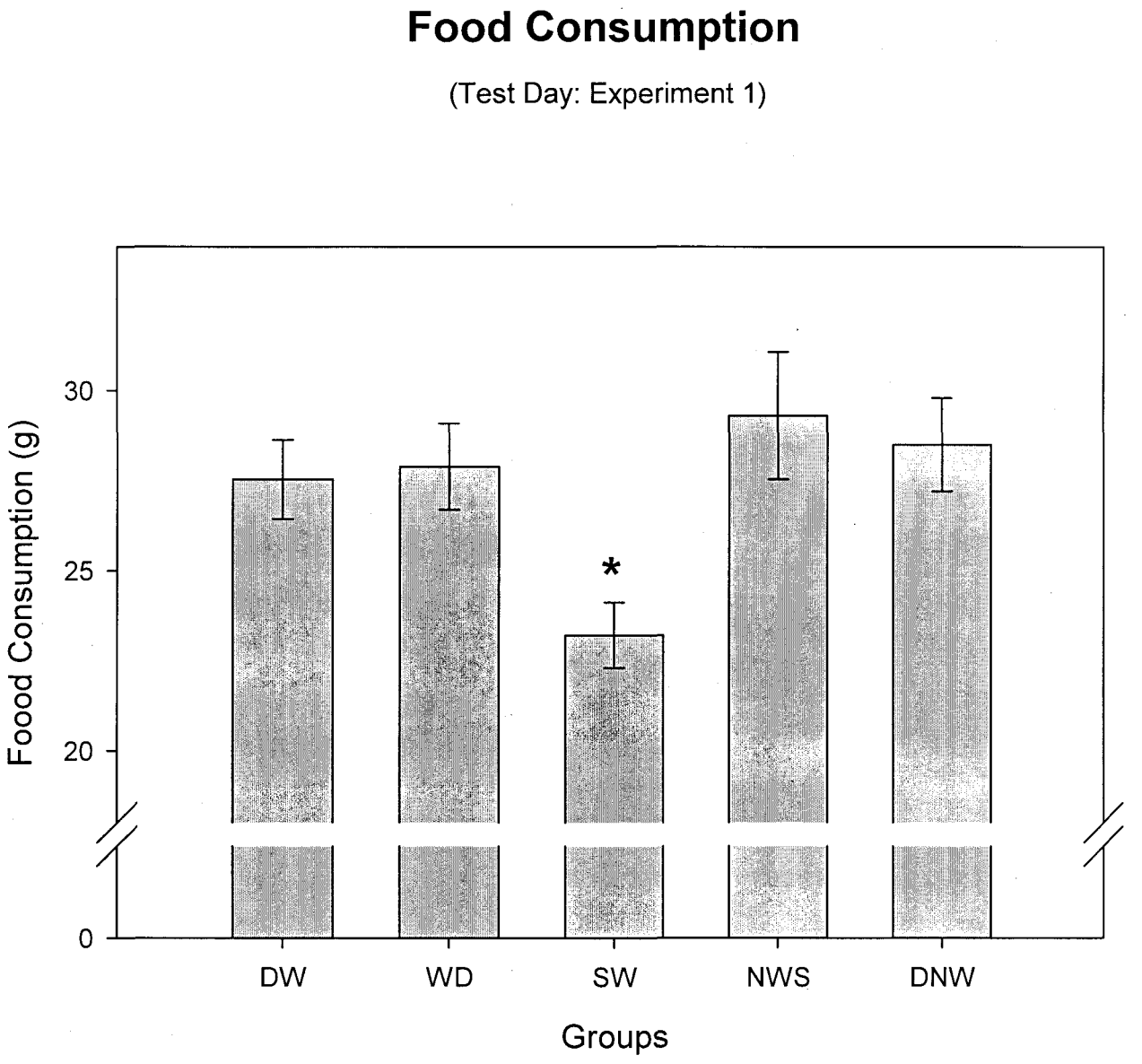

Food Consumption
(Test Day: Experiment 1)
Foood Consumption (g)
30
25
20
0
*
DW
WD
SW
NWS
DNW
Groups

Figure 5

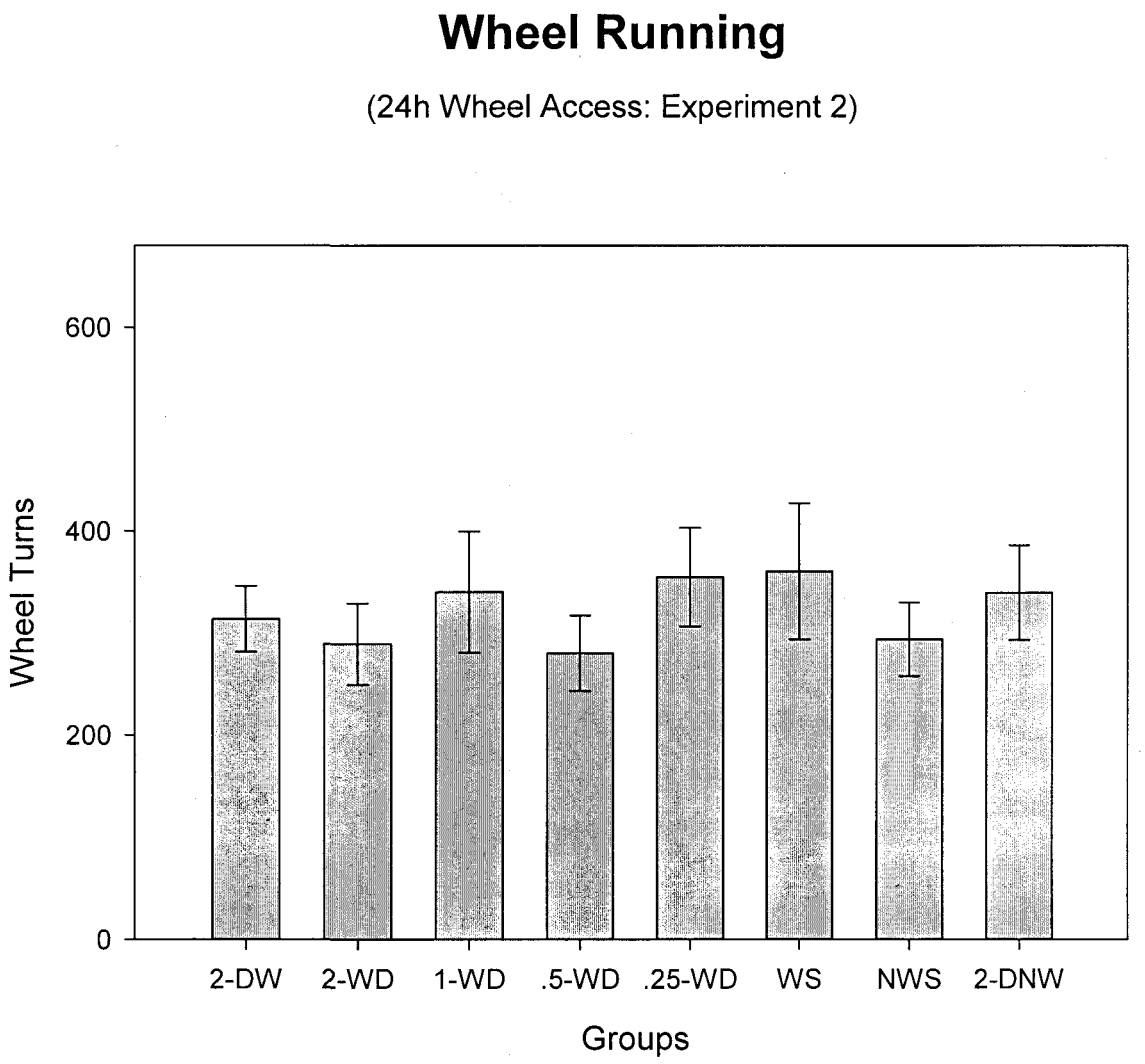

Wheel Running
(24h Wheel Access: Experiment 2)
Wheel Turns
600
400
200
0
2-DW
2-WD
1-WD
.5-WD
.25-WD
WS
NWS
2-DNW
Groups

Figure 6

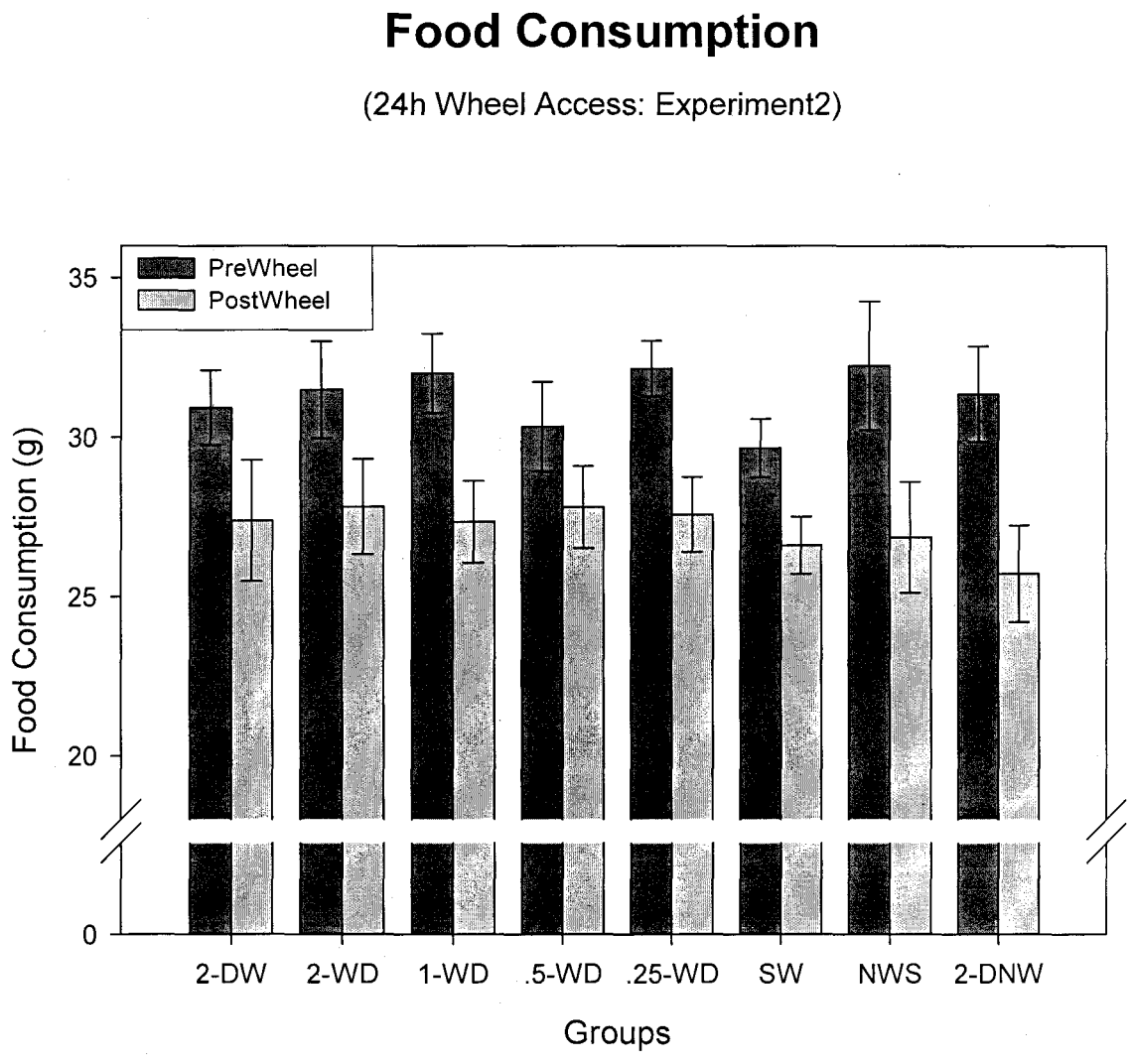

Food Consumption
(24h Wheel Access: Experiment2)
PreWheel
PostWheel
Food Consumption (g)
35
30
25
20
0
2-DW
2-WD
1-WD
.5-WD
.25-WD
SW
NWS
2-DNW
Groups

Figure 7

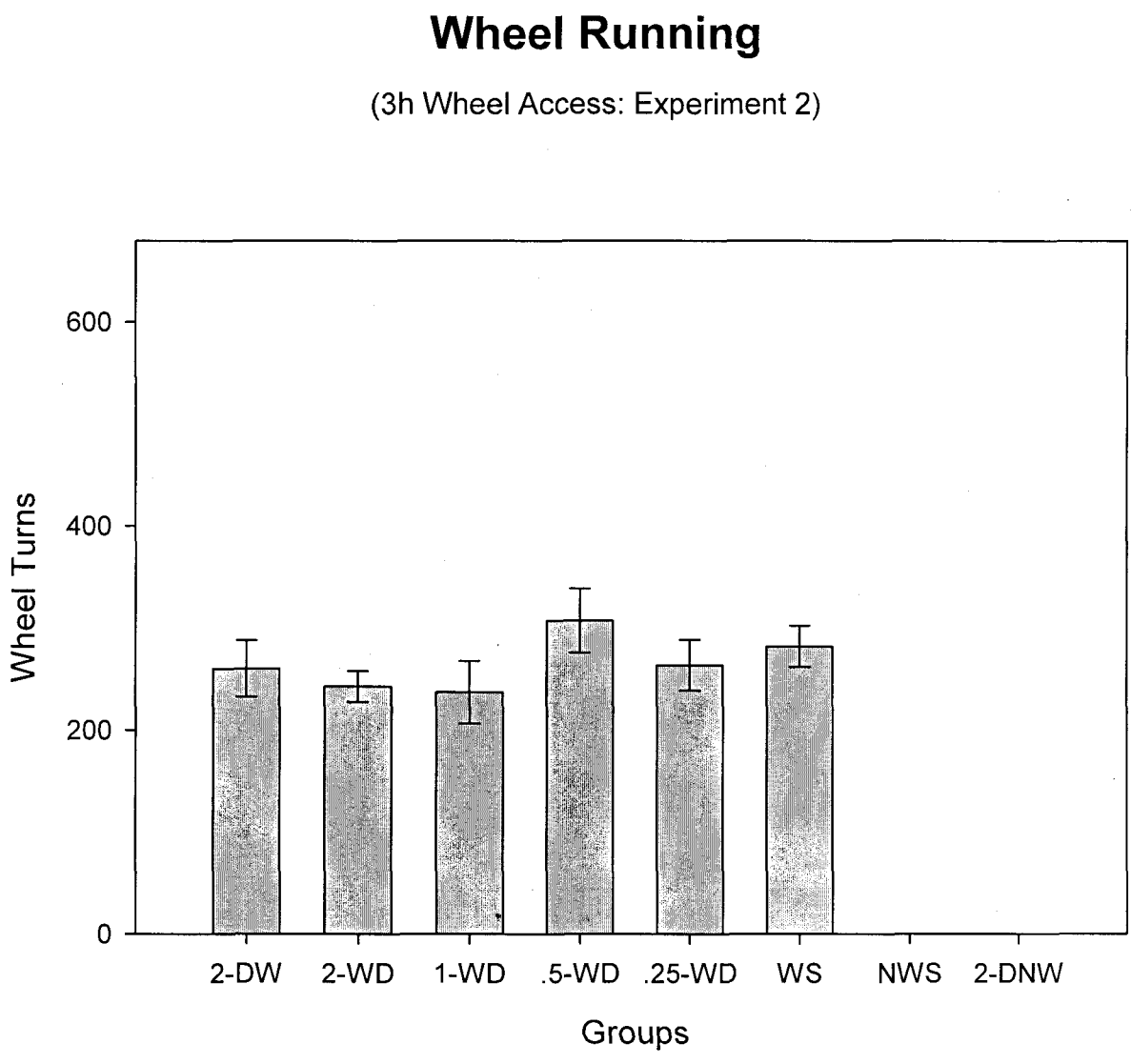
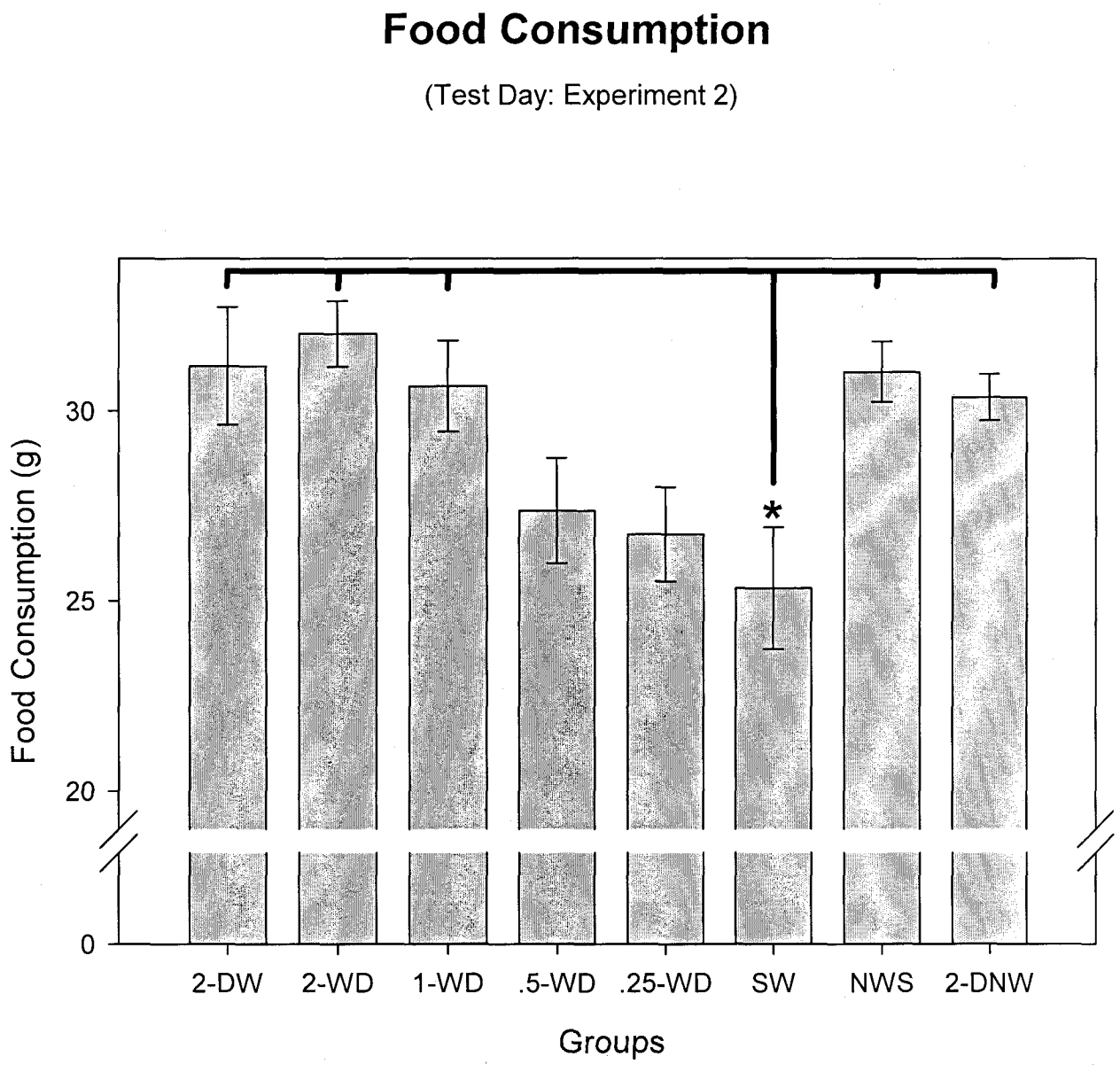

Wheel Running
(3h Wheel Access: Experiment 2)
Wheel Turns
600
400
200
0
2-DW
2-WD
1-WD
.5-WD
.25-WD
WS
NWS
2-DNW
Groups

Figure 8


Food Consumption
(Test Day: Experiment 2)
Food Consumption (g)
30
25
20
0
*
2-DW
2-WD
1-WD
.5-WD
.25-WD
SW
NWS
2-DNW
Groups