2013

The Effects of Housing Manipulations on Wheel Running, Feeding and Body Weight in Female Rats

Angela Mastroianni
Wilfrid Laurier University, mast7710@Mylaurier.ca

The Effects of Housing Manipulations on Wheel Running, Feeding and Body Weight in Female Rats

By

Angela Mastroianni

Bachelor of Science (Honours), Psychology,

University of Toronto, 2008

THESIS

Submitted to the Department of Psychology

in partial fulfillment of the requirements for

Masters of Science, Behavioural Neuroscience

Wilfrid Laurier University

Abstract

Providing rats with running wheel access results in a short-term reduction in feeding and body weight relative to controls; known as the wheel-induced feeding suppression (WIFS). WIFS may parallel aspects of anorexia nervosa, an eating disorder that mostly affects females. Yet, most studies of WIFS and related models use male rats. The present study included female and male rats, where half were given wheel access to measure effects on feeding and body weight. Replication 1 females and Replication 2 males were in unisex housing. Replication 3 males and females were housed in same room. Rats were individually (IH) or pair housed (PH) as it may reduce stress and the WIFS. None of the replications found evidence that pair housing changed the WIFS. Females in Replication 1 had a shorter WIFS than Replication 2 males. In Replication 3, the WIFS was similar in both sexes. Pair housing affected wheel turn counts. Female PH rats ran in the wheel together, resulting in fewer wheel counts than for IH rats. PH males did not run together, resulting in equal or higher counts than IH males. Female wheel running and feeding were affected by the estrous cycle; running was elevated and feeding was reduced at estrus. The Whitten effect suggests that males can induce estrous cycle regularity and synchrony. In Replication 3, males induced estrous regularity and synchrony as seen in the wheel running and feeding of the females. The absence of males in Replication 1 led to reduced estrous regularity and synchrony. It is concluded that exercise has immediate and lasting effect on energy balance. Females regulate this energy balance differently than males, a difference possibly driven by gonadal hormones and by a number of factors in the environment. The sex differences in regulating energy balance can have important implications for eating disorders that are sexually dimorphic, like anorexia and even those of increased consumption, such as obesity.

Keywords: wheel running, pair housing, feeding, weight, sex differences, estrous cycle

Table of Contents

Acknowledgements

I would like to thank Dr. Rudy Eikelboom for going above and beyond throughout this process and for exceeding my expectations as an advisor. Despite being pulled in various directions and the daunting piles of responsibility, he made me and this thesis his priority. I appreciate the patience he put forth, the guidance and wisdom, as well as the thorough feedback that came with swift speed (even if it meant secretly editing when on 'vacation'). A seemingly impossible task became possible because of his support. I am grateful to have had the opportunity to begin my academic journey in the Eikelboom lab.

I would like to thank Diano Marrone, Paul Mallet and Elena Choleris for agreeing to be a part of my committee and for their contributions to both the document and my knowledge base. I appreciate all of the help that came from my lab mates, who assisted me and provided useful feedback from beginning to end.

I was able to make it through maintaining my sanity (at least from my perspective) because of my support system. I am thankful for my friends (especially my neuro crew) and their endless cheerleading, 'handling' and motivational words over the past two years. To Wes: thank you for being my rock and for motivating me when I needed it most. You felt every bump along the MSc-way but always carried me through. To my family: thank you for your infinite cascade of love and support. I have amazing parents and sisters, and I could not be luckier.

Introduction

Wheel running has been extensively studied in rats since the late nineteenth century (Stewart, 1898; Sherwin, 1998). Studies have examined the variables that affect the patterns and the amounts of wheel running. Work has also looked at the many consequences that exercise, such as wheel running, has on other behaviours, including effects on physical and mental health. The impact of exercise on health suggests that understanding wheel running patterns in rodents may have useful implications for humans (Eikelboom, 1999). Exercise is also an important factor in the eating disorder anorexia nervosa (AN) as high levels of exercise are seen prior to its onset, and also once AN has developed, excessive exercise has been said to maintain it (Beumont, Beumont and Touyz, 1996). Understanding what drives these abnormally high levels of physical activity in AN becomes important. Conversely, low levels of exercise have been suggested as a causal factor in daunting health issues like obesity (Barlow et al., 2012). Sedentary lifestyles can lead to obesity, heart disease, diabetes and many other illnesses, including mental disorders. Many of these lifestyle issues and the prevalence of their medical consequences are differentiated by gender. AN mostly affects women (Lau et al., 2007). Although influenced by culture, global obesity rates are higher for women (14%) than men (10%) (World Health Organization, 2008).

Higher levels of physical activity naturally imply increased energy expenditure. Following exercise, it might intuitively be expected that caloric intake should increase, preventing body weight from dropping below healthy levels. However, in rats, a short-term reduction in feeding is typically seen when a wheel is made available and the animals show increasing levels of running. This phenomenon, known as the wheel-induced feeding suppression (WIFS), is also accompanied by an immediate loss of body weight that is maintained as long as

the rats have wheel access (Looy and Eikelboom, 1989; Afonso and Eikelboom, 2003). It appears that the opportunity to run changes energy balance over the longer term. These findings have implications for a better understanding of the relationship between exercise, food intake and body weight. Not only this, the WIFS may provide insight leading to a better understanding of AN. Both AN and WIFS may be driven by an excessive level of exercise. Thus, WIFS has been suggested as an animal model of AN (Lattanzio and Eikelboom, 2003). Animal models that explore the biological substrate of medical disorders, including WIFS for AN, can be useful when looking at causes, prevention and treatment for these problems (Siegfried et al., 2003).

One concern in exploring these models is that important sex differences exist in eating disorders and in the animal behaviours that we rely on to model the disorder. Despite the female predominance in human eating disorders, most research using animal models has been done with males. It is important to note that aspects of wheel running and their outcomes are sexually dimorphic in rats (Anantharman-Barr and Decombaz, 1987). For example, males tend to run at much lower levels than female rats (Eikelboom and Mills, 1988; Wang, 1923). Despite these differences, most research fails to test both females and males. Studies examining wheel running have been carried out for over 100 years, yet female studies and comparisons are rare. Male wheel running may be relatively understood after a century of work, but less is known about female wheel running. Studies that include females are needed as many of the exercise-related eating disorders, such as AN, an often deadly illness, mostly affects females (Davis, 2007). Work that neglects comparisons between both sexes hinders progress in understanding female-driven eating disorders.

The focus in this thesis will therefore be on wheel running in female rats, as this has implications for specific human health issues that are sexually dimorphic. It has been suggested

that exercise plays an important role in eating disorders that result in an unhealthy body weight (Davis, 1997). Female adult Sprague Dawley rats were used and compared to males in order to explore: wheel running as behaviour, the sex differences in the consequences of wheel running and some factors (hormonal and social) that may impact on the levels of running.

Wheel running behaviour

Typical running patterns in male and female rats

Male adult rats initially begin running about 1000 wheel turns a day and escalate their running levels rather quickly. Upon reaching a plateau (after roughly 15 days of wheel access), adult male rats exhibit an average running level of 5-10 kilometers or about 5000-10,000 wheel turns a day (Anantharman-Barr and Decombaz, 1987). Note that the circumference of a typical running wheel used in most research is about 1 meter or 0.001 kilometers, so 1000 wheel turns are roughly 1 kilometer. Once the plateau in running has been reached, male adult rats tend to stabilize their running levels for the remainder of the wheel access period, with intensity decreasing slowly with age (over 7 to 8 months) (Mondon et al., 1985).

Female rats acquire the behavior of wheel running more quickly than males. They tend to run further, faster and longer, and show hormone-induced changes in running (Wang, 1923; Eckel et al., 2000). Female adult rats run at much higher levels than males and average 10 kilometers of running per day (Cortright et al., 1997). For example, a pilot study was done with 32 female and male pair housed rats, where half were given wheel access. Wheel turns were recorded for 30 days and showed a marked sex difference in wheel running: the males did not exceed 7,000 wheel turns a rat per day, whereas the females approached a maximum of over 20,000 wheel turns in the experiment (Mastroianni, 2011 unpublished data). Females

demonstrated less stable levels of running than males with large day-to-day fluctuations, even post plateau. This sex difference in running is typical of past studies, with females fluctuating and greatly exceeding the running level of males (Eikelboom and Mills, 1988). Female wheel running is affected by the estrous cycle (explaining the fluctuations), with wheel running markedly increasing during the night of estrus and decreasing during metestrus (Eckel et al., 2000). Male wheel running does not show obvious hormone–induced changes (Eckel, 2011).

Although several aspects of running show sex differences, running patterns in males and females show some parallels. Both female and male rats do the majority of wheel running at night (more so at the beginning of the dark), their active phase of the circadian cycle. In fact, rats do not generally exhibit significant locomotion activity or eating during the light or inactive cycle period. Females show higher wheel turning counts due to longer running durations, increased number of wheel turns per running period and higher speed compared to males (Eikelboom and Mills, 1988). Altogether, it is clear that distinct sex differences in wheel running exist.

Wheel running as a reward

It is well known that rats will voluntarily run in wheels and that they find this behaviour rewarding. Rats, upon wheel access, will increase wheel running over days to very high amounts, which suggests that it is self-reinforcing. Physical exercise has been shown to activate the mesolimbic dopamine system and, when stimulated, causes increases in voluntary wheel running (Schwarzberg and Roth, 1989).

This increase in running is unlike the change in locomotion seen in standard shoebox cages or circular runways, which decreases overtime (Eayrs, 1954; Koh et al., 2000). Rats will press a lever in order to gain wheel access (Kagan and Berkun, 1954), which is indicative of its

reward value (Robinson, 2004). They will work to unlock a wheel to be able to run (Kagan and Berkun 1954; Tepper and Weiss 1986; Collier et al., 1975). Additionally, rats display a conditioned place preference if an environment is paired with a running wheel (Lett et al., 2000). The length of daily wheel access can determine if rats' running escalates to high levels. Lattanzio and Eikelboom (2003) found that rats given 2 hours of daily wheel running access show low and stable running, which at an average of 350 wheel turns per day. Rats given longer 4 hour daily access showed typical escalation in running that approached an average of 3000 wheel turns per session.

Relevance for humans

As previously mentioned, maladaptive exercise in humans can have harmful consequences on physical and mental health. Unhealthy amounts of exercise can be linked to eating disorders of reduced intake, namely AN.

Anorexia Nervosa

As AN can be deadly, extensive research has attempted to uncover its cause. Characterized by severe weight loss due to extreme food reduction, AN has the highest mortality rate of any mental disorder (American Psychiatric Association, 2000). The Public Health Agency of Canada has found that 5-20% of AN patients die from complications of the disorder (Public Health Agency of Canada, 2005). A defining feature of this disorder is the maintenance of a body weight that is less than 85% of what is normal for one's optimal Body Mass Index (American Psychiatric Association, 2000). Individuals with the disorder restrict themselves to small amounts of food due to an irrational fear of gaining weight (Nogal and Lewiński, 2008).

AN is characterized by a distorted body image that drives starvation; AN patients allow themselves an average of 600 to 800 calories per day (Frude, 1998).

Although the average duration of the disorder is five years, it lasts six to ten years in 31% of patients and eleven to fifteen years in 16% (Sullivan, 1995). Studies estimate that 5 to 10% of anorexic individuals will die within 10 years of the onset of the disorder, 18 to 20% of anorexics die after 20 years (Sullivan, 1995). Given its high prevalence and mortality rate, understanding the etiology is especially vital. Full recovery is only seen in roughly 30 to 40% of patients (American Psychiatric Association, 2000), demonstrating that improved treatments are needed.

Excessive Exercise in AN

For many decades, clinicians have noted that anorexics often undergo starvation and excessive exercising simultaneously. It is common for AN patients to demonstrate a high drive for physical activity, which tends to result in a compulsive need to exercise. Excessive exercise during an acute phase of the disorder has been seen in 80.8% of AN patients (Davis, 1997). Not surprisingly, it has also been found that up to 80% of anorexics have been described as having hyperactive personality characteristics (Davis, 1997). As a result of the link between high levels of physical activity and AN, exercise is considered a major contributing factor to the onset and maintenance of the starvation symptoms.

Several other factors are important in the etiology of AN, some of which are genetic factors, social and cultural influences (highest incidence is in Western societies), socio-economic status, and the presence of early adversity. There are neurobiological factors that may predispose certain individuals or worsen symptoms of AN (Siegfried et al., 2003); one of which may be a high drive for physical activity. In addition, neurobiological changes are likely occurring after the onset of AN. Despite the identification of several important variables implicated in the

etiology of AN, a sole cause is likely not responsible. Its onset appears to be influenced by multiple factors that interact in a complex manner (Garner and Garfinkel, 1997). As anorexia is a multifaceted mental disorder, it may be difficult to understand its onset in order to prevent or treat it. However, the excessive exercise feature is important and may link animal work on wheel running to eating disorders like AN.

Animals are not explicitly impacted by the social pressures that humans experience to achieve a certain (low) body weight in order to be perceived as physically attractive. Despite this, a feeding suppression can be induced in animals, which suggests a neurobiological component to self-starvation as a consequence of high levels of exercise. Animal models suggest that introducing intense exercise may have profound and immediate effects on energy balance and, in turn, food intake levels. It is important to better understand how exercise can affect feeding. Consequently, animal models of anorexia provide a basis for exploring the neurobiological changes that occur as the disorder progresses. They also allow for the testing of possible treatments (Siegfried et al., 2003).

In cases where excessive exercise is central to the AN onset and maintenance, the etiology may be the result of biological processes triggered by ideologies from culture for diet and exercise (Epling and Pierce, 1988). AN is the only eating disorder that requires a biological component for its diagnosis, which is the presence of amenorrhea (Davis, 1997). Age of onset furthers this point, as this disorder manifests only after the onset of puberty (Patton et al., 2003). Sex differences in the prevalence suggest that important hormonal factors may be at play (Woodside et al., 2001). Work looking at twins and relatives has found a familial link in the etiology, highlighting the importance of biological and genetic factors in AN (Bulik et al., 2000). Recent work is leading towards the view that biological predispositions may be involved

in most AN cases as genetics may account for up to 70% of risk of developing the disease. Focus has been on examining neurobiological mechanisms in AN patients, such as alterations in serotonin functioning, a neurotransmitter involved in mood and appetite, as well as opioid receptors involved in appetitive, food intake and energy regulation (Clark et al., 2012). Neurobiological contributions to the disorder require more attention as they are not fully understood.

Wheel effects on feeding

Two animal models will be highlighted that may provide insight into energy balance following exercise. They have also been proposed as animal models of AN (Epling and Pierce 1988; Lattanzio and Eikelboom 2003) as individuals with AN often simultaneously starve and exercise excessively. This reduced feeding level and excessive exercise in AN parallels these animal models that examine food intake and body weight following wheel running in rodents.

Activity-Based Anorexia (ABA) Model

A well-known and commonly utilized animal model of AN is the ABA model. It provides insight into how exercise can affect food intake and weight. In a typical ABA experimental design, rats or mice are placed on a food restriction and provided access to a running wheel and compared to non-running controls (Routtenberg, 1968). Food intake is restricted to one brief daily feeding period, usually for 1 hour, and access to a running wheel is provided for the remainder of the day, usually for 23 hours. During food access periods, rats are removed from their wheel cages and placed in a standard cage. Control animals are given an equivalent food restriction but no wheel access. Food consumption is compared between running and non-running groups. ABA experiments find that rodents not only run at high levels, but they

reduce their food intake compared to non-running controls. Most rats in these experiments self-starve to the point of death, despite having enough access to food for control rats to survive with no problems (Epling et al., 1983). Rodents will drop to 70% of their normal body weight and develop gastric stomach ulcers prior to death (Doerries et al., 1991). This is counterintuitive; should an animal increase energy expenditure through running, food intake should increase as a result. However, the intense running (paired with food restriction) appears to induce self-starvation in the rodents, relative to non-running controls. This is reminiscent of the high levels of exercise seen in many AN individuals and some do in fact starve to death. The fact that rats voluntarily self-starve does not seem to be strategy to increase wheel running time by spending less time eating. In this model, rats are taken away from the wheel for their feeding period and still choose eat very little. Unlike some anorexics, rats fully recover their feeding levels and body weight should they be removed from the procedure prior to ulcer development (Boakes et al., 1999). If they are kept in the procedure, they generally will not survive.

In the ABA model, few studies have examined females and those that have, have reported conflicting results. Paré et al. (1978) found that females were more vulnerable than males (faster mortality), while Doerries et al. (1991) found that males were more vulnerable, losing weight more rapidly than females. Boakes and colleagues (1999) report that males and females matched for age at 41 or 41 days, but not weight, showed no ABA sex differences. Females that were 136 days old and matched for weight with the young males showed a slower body weight loss compared to both the younger females and the males. Boakes suggested that older adult females may be less vulnerable because they are quicker to learn the restricted feeding schedule compared to males. Mixed results may be due to differences in procedure, age of the animals and

also some studies either matched age or weight between males and females and this choice has a significant effect.

A disadvantage of this model is that it results in the mortality of most rats used in the study. Additionally, the rodents need to learn that food is only available during specific times. Not only is there a learning component that may influence food intake, a restricted food diet is in itself stressful. It is not clear if the self-starvation is a result of the wheel or the wheel's affects in combination with the stress/learning that food restriction involves. Rats that are pre-exposed to the restricted feeding schedule prior to wheel access will be more likely to survive than rats without this experience (Routtenberg, 1968). Rats that are introduced to the ABA model, allowed to recover and then re-introduced to the procedure multiple times show increased food intake and body weight compared to rats without prior ABA experience (Hampstead et al., 2003). Food access in the ABA model is commonly offered during the rats' light cycles, when they are inactive and this may reduce feeding levels as well. Much of the research is done in male rats, although some have begun working with females in order to study the sex differences in this model and AN (Eckel et al., 2000). Despite its limitations, the ABA model offers evidence that exercise can have an important paradoxical impact on energy balance, which may be fatal for some.

Wheel-induced Feeding Suppression

A wheel-induced feeding suppression (WIFS) model of anorexia is an alternative animal model that also examines the impacts of exercise on feeding and weight in rodents (Looy and Eikelboom, 1989; Mueller et.al, 1997; Lattanzio and Eikelboom, 2003; Afonso and Eikelboom, 2003). The main differentiator between the WIFS and the ABA model is the use of *ad libitum* food for all rats in the WIFS, which prevents the mortality of the animals. This model provides

unlimited wheel access to the rats, which are compared to non-running controls. This model has found that running rats, even with daily food access, will still reduce their food intake up to 25%-37% relative to control rats. This feeding suppression has been shown to last anywhere from 7 to 12 days (Afonso and Eikelboom, 2003). After the feeding suppression period, feeding gradually recovers and, in some cases, surpasses that of controls. However, unlike the ABA model, the WIFS model allows one to see the feeding patterns that occur after suppression recovery. It is important to note that all the rats do recover from the feeding suppression, yet some patients with AN do not recover from their self-starvation behaviours. Despite recovered feeding patterns, running rats experience an initial drop in body weight compared to non-running controls. The difference in body weight remains until wheel access is removed. Only then does body weight recover to control levels (Afonso and Eikelboom, 2003). This model can be linked to AN, as some clinicians have recommended reducing excessive exercise levels to supervised, reduced and healthier levels as a form of treatment (Beumont et al., 1994).

Although unlimited wheel access induces short-term reduced feeding (a maximum of 12 days), even a two-hour period of wheel access is sufficient to provoke a feeding suppression over the following night (Lattanzio and Eikelboom, 2003). Even with only two hours of daily wheel access, rats run a substantial amount, roughly 350 wheel turns. While it is well established that rats are often high runners and make considerable efforts to gain wheel access, there is considerable variability between rats in the levels of running (Eikelboom, 1999). The variability in running does not correlate with the degree of feeding suppression. In other words, high and low running rats will not demonstrate differences in the WIFS severity or duration (Afonso and Eikelboom, 2003).

WIFS studies have introduced wheel access to both weanling and adult rats in order to identify potential developmental or age effects. Introducing Sprague Dawley rats to the wheel before puberty, <42 days, does not result in a WIFS (Dalton-Jez, 2006; Cortright et al., 1997). The mentioned pilot study also replicated this finding (Mastroianni, 2011 unpublished data). Weanling wheel rats and control rats have similar feeding and body weight. However, if the wheel is introduced after puberty, in adulthood, the feeding suppression is seen (Afonso and Eikelboom, 2003). This finding parallels the human AN literature in that the vulnerability for onset is typically seen after puberty. Individuals between the ages of 15 and 18 years of age are most susceptible to AN (Patton et al., 2003), making adolescence a vulnerable developmental period for onset. In fact, AN is the third most common chronic illness among adolescents (Strober and Freeman, 1998).

Sex differences have also been examined in a few studies looking at the WIFS. Most work exploring the WIFS has been done with males as females show estrous cycle driven changes in feeding and running. Nonetheless, the WIFS has been seen in both sexes (Dalton-Jez, 2006; Afonso, 2000). However, some researchers suggest that running does not affect females or that the WIFS in females is not always as lasting compared to what is seen in males (Dalton-Jez, 2006; Afonso, 2000). WIFS results in males are usually robust, pronounced and last 7-12 days. For example, Afonso and Eikelboom (2003) found an eight day feeding suppression in males and a six day feeding suppression in females. The pilot study done with female and male rats housed in same-sex pairs did not show a distinct sex difference in the WIFS. Both sexes had four-day suppressions with similar initial drops in feeding from baseline (Mastroianni, 2011 unpublished data). It is possible that the short WIFS may have resulted from the social interaction offered by pair housing. However, there were sex differences in feeding after the initial period of the WIFS.

Once the male group recovered from the feeding suppression, there was no compensation or increased eating following the first few days of wheel access. They matched the feeding level of the non-running control rats and only very slightly elevated their feeding. The females, on the other hand, show a marked elevation in feeding after the first few days of wheel access, suggesting that the females were compensating for the increased calorie expenditure due to running. These results seen with males are unlike the results seen by Afonso and Eikelboom (2003) who found that wheel running males showed elevated feeding post WIFS recovery, relative to controls. The food elevation was not as high as that seen in females.

Explanations for the sex differences remain unclear, as are the inconsistencies seen in female WIFS. Gender is an important variable in human eating disorders, as 90-95% of AN cases are female (Strober and Freeman, 1998). Cases are reported in males and appear to have increased in recent years. However, AN still affects females ten times more than males, suggesting that it is mostly a female disorder (Woodside et al., 2001). The prevalence among females is rather high, anywhere from 0.5 to 3.7% of adolescent and young women meet the criteria for AN (Woodside et al., 2001).

It is important to note that exercise itself can improve health for humans and animals. It has been shown that exercise extends life span in animals and is linked to many cognitive and neurological benefits for humans (Fletcher et al., 1996). It is when exercise becomes excessive and is then paired with unhealthy reduced food intake that it can become a problem. This is true for humans and animals alike. Of course, extended periods of being sedentary paired with excessive food intake can also be a problem. This lifestyle may be an issue in the animal studies for the non-running controls who live with *ad libitum* food with no real opportunity to engage in normal behaviours that involve energy expenditure (Boggiano et al., 2008).

Variables involved in wheel running

Given that wheel running levels can vary considerably across studies, it is important to understand the factors that drive the differences. Factors in a rats' environment can have an effect on feeding and wheel running, particularly in adult female rats, which in turn may affect body weight. In females, running can be affected by internal hormonal changes driven by the estrous cycle (Wang, 1923; Eckel et al., 2000). External factors, the presence of other females or males, can affect the estrous cycle in female rats (McClintock, 1978) and, perhaps as a consequence, levels of running. Additionally, stress can have important effects on wheel running, feeding and, consequently, body weight. Changes in the level of stress are affected by social factors, such as a rats' housing environment (Vallès et al., 2012; Baldwin et al., 1995; Ruis et al., 1999).

Internal hormone effects

Female wheel running has been shown to be influenced by stages of the estrous cycle (Wang 1923; Beatty, 1979; Anantharaman-Barr and Decombaz, 1987). The female estrous cycle consists of four stages over 4 to 5 days: metestrus, diestrus, proestrus and estrus. Locomotor activity is lowest during diestrus, which typically lasts 55 to 57 hours, and highest on the night of estrus, which lasts 25 to 27 hours. On the night of estrus, females, if given wheel access, have been shown to elevate running by over 100% from levels seen in diestrus (Eckel et al., 2000). This elevated activity may assist females in actively seeking males as, during the period of late proestrus and estrus, known as the period of 'heat', the female rat is sexually receptive and fertile. Estrogen levels, specifically estradiol, peak during pro estrus and reach lowest levels just prior to estrus. Defined by vaginal cytology, the start of estrus is defined as the point when cells

show a progressive leukocyte infiltration (Westwood, 2008).The estrous cycle also affects feeding patterns. Feeding and body weight have been shown to be reduced during the night of estrus and highest during diestrus (Wang, 1923; Eckel et al., 2000). This reduction in feeding during estrus is caused by a reduced meal size, as the frequency of meals either remains static or may even increase (Eckel et al., 2000). Providing female rats with access to a running wheel does not change the estrus-induced feeding suppression, as wheel running and non-running females show similar reductions (Eckel et al., 2000). Estrus in rats is thus clearly marked by reliable changes in two behaviours; feeding is reduced and running is increased when the rats are in heat.

Effects from other females

Since the female estrous cycle has effects on wheel running and feeding, it is important to understand the mechanisms that alter or change the estrous cycle. The cycle of the female rat can be susceptible to change based on environmental influences. A change may alter the duration of the cycle, as well as the timing of estrus initiation. One such factor that can influence both the duration and phase of the cycle is the close proximity to other female rats. All-female social grouping can cause a synchronization of the estrous cycle as female rats will come to experience estrus simultaneously (McClintock, 1978). Before grouping, females had diverse cycles that had estrus occur on different nights but after living together, their cycles became synchronous. Simultaneous estrus in a group of female rats is a seemingly adaptive phenomenon as rats in social contexts tend to engage in group mating, inter changing partners (McClintock et al., 1982). If estrus occurs simultaneously, male rats engage in copulation with multiple females in an optimal manner, thereby increasing the chances of successful reproduction. Another possibility is synchrony can enable communal pup rearing among groups of female adults (Ims, 1990). Other

studies have seen evidence for estrus synchrony in rats, humans, mammals and non-human primates, hamsters, and possums (Schinckel, 1954; Shelton, 1960). It appears that the mechanism driving the proximity effect is olfactory-driven and is a result of volatile, airborne chemicals. Physical contact is not necessary for the synchrony to occur, however specific close proximity is necessary (McClintock, 1978).

Cycle synchrony among females in close proximity has been shown in humans as well. McClintock (1971) tracked the menstrual cycle in 135 students living in a women's college. She compared the menstrual cycles for close friends, who indicated seeing each other often, and random pairs. She found a significant increase in cycle synchronization among roommates and close friends over the term.

Effects from males

The presence and absence of males can affect the regularity and synchrony of the estrous cycle in female rodents. Since the mid 1950s, it has been known that male presence can induce synchronous estrous cycles in female mice. Known as the Whitten effect, it is an adaptive mechanism that enables optimal reproduction in a given group of mice (Whitten, 1956). Conversely, the estrous cycle has been shown to be prolonged or suppressed without male presence in female mice (Van der Lee and Boot, 1956). A healthy or normal estrous cycle has duration of 4 days in rodents. However, an absence of males in the environment will lengthen the cycle; the Lee-Boot effect. This effect is due to inhibited secretion of follicle-stimulating hormones, which delays estrus (Van der Lee and Boot, 1956). Upon re-introduction of males, the estrous cycle in female mice will again become regular and synchronous (Whitten, 1959). McClintock's (1971) work suggests that cycle regularity can also occur in humans; she asked the participants to report how often they were exposed to males per week. Females who reported

seeing males less than three times per week tended to have a significantly longer than 28-day cycle (McClintock, 1971). Some investigators have suggested that the Whitten and Lee-Boot effects are absent in rats, while others argue it is present (Cooper and Haynes, 1967; Hughes, 1964) or present with a more subtle effect in female rats, suggesting that they may be less sensitive to the presence of males.

Similar to the effects of close proximity on female cycles, the male effect on the female estrous cycle does not require physical contact. The male must be positioned nearby perhaps so that the odours, potentially from the urine, or ultrasonic vocalizations coming from the males can reach the females. Whitten and Bronson (1968) found how the female mice were positioned in relation to the males affected synchrony, and certain proximity was required. They suggested that volatile airborne chemicals from the males were responsible and provide a signal to female olfactory systems (Whitten et al., 1968).

Interactions in food intake, exercise and stress

Stress can have complex effects on locomotion, feeding and body weight. Stress is a construct that is difficult to define as "stressors" differ in their specific effects, do not always elicit the exact same response, and may vary across individuals. Stress can have negative or positive consequences for one's health and, in acute situations, has physiological effects that activate what has been called the fight-or-flight response. Hans Seyle (1976) broadly defines stress as "the nonspecific response of the body to any demand, whether it is caused by, or results in, pleasant or unpleasant conditions".

It has been found that food intake can be highly sensitive to the presence of stressors (Martí et al., 1994), in both rodents and humans. In rats, moderate stressors can cause a reduced food intake for 24 hours after presentation and severe stressors can induce a long-lasting

suppression (up to 9 days) (Vallès et al., 2012). The stressor of early maternal separation exasperates anorexia symptoms in the ABA model in female and male rats, when compared with maternally handled rats (Hancock et al., 2009). Food access has been shown to affect level of wheel running. Rats that are food deprived show increased levels of running (Richter, 1922; Gross, 1968). Rats that are stressed (cognitively or physically) will run more, but the ability to engage in long term running can also reduce the impact of stress by reducing stress hormone levels after 8 weeks of exercise (Sasse et al., 2008). In humans, it has been found that anorexic patients have higher cortisol levels compared to healthy controls; cortisol levels in humans are a physiological index of stress. The elevated cortisol levels return to 'normal' with recovery from AN (Walsh et al., 1981). It is not clear if the changes in cortisol levels stem from the reduction in food intake (a starvation effect), general stress in AN or the elevated physical activity associated with this disorder.

It is known that wheel running reduces food consumption in rodents temporarily. However, exercise can have effects on short-term intake in humans as well. King and colleagues (1994) assigned males to low, high intensity or no exercise conditions in a study of appetite. Motivation to eat was measured using hunger scales and measured by the onset of eating after different levels of exercise. They found that intense exercise males had a short-lived reduction in motivation to eat and delayed the onset of meals, creating a temporary negative energy balance. Thompson and colleagues (1988) completed a similar study with men and found that, although hunger was briefly suppressed in their high-intensity exercise condition, intake of liquid-sources of calories was higher after exercise.

Exercise, activity and stress clearly have complex interactions. Exercise activates the stress-related systems in the brain as it acts on the hypothalamic-pituitary-adrenal (HPA) axis,

releasing corticosterone (or cortisol in humans), which in turn impacts other stress responses and digestion. It initially hyper-activates the HPA axis, which may contribute to the short-term feeding suppression seen after wheel running access (Drost et al., 2007). In rats, wheel running has been viewed as a rewarding, controllable stressor affecting homeostasis but not posing immediate threat to rodents (Stranahan et al., 2010).

Sex differences in stress responses

It is important to be aware that there are sex differences in reactions to various stressors. For example, female rats are more sensitive (show bigger stress response) to the introduction of a novel chronic stress than males, but are better able to cope (smaller stress responses) if the stressor is repeated (Dalla et al., 2005). In other words, males tend to elevate their stress levels in response to repeated stressors, while female rats tend to adapt. With humans, women appear more susceptible than men to stress-related mental illness, such as severe depression (Dalla et al., 2005).

Housing effects on stress and feeding

Animal housing conditions can have an effect on tolerance to stress, and in turn may affect appetitive behaviours (Martí et al., 1994). Individually housing rats does not mimic a natural environment, as they live in colonies and are social creatures in the wild (Boggiano et al., 2008). Studies have shown that social isolation causes adverse effects in laboratory animals (Boggiano et al., 2008) and isolation housing is not an optimal environment for the general health of rats. In some rodent species, social isolation can activate the HPA axis, making the animals more sensitive to the effects of other stressors (Baldwin et al., 1995; Ruis et al., 1999). In group-housed rodents, corticosterone release is reduced, presumably resulting in better HPA

axis feedback (Ruis et al., 1999). Pair or group housing rats can increase tolerance to stressors such as injections, relative to individually housed rats (Vallès et al., 2012).

It may be reasonable to explore the possibility that housing manipulations such as pair/group housing, may serve to mitigate stress and in turn prevent or suppress the WIFS. Boakes et al. (1999) found that female and male rats housed in groups of four are better able to adapt to restricted feeding schedules in the ABA model than are individually housed rats. Note that both females and males benefited from these housing manipulations to the same degree. When introduced to a running wheel and put on a restricted food diet, group housed rats benefited by having a higher body weight than individually housed rats. This is presumably because group housing reduced stress levels, allowed rats to learn the feeding schedule and better survive. The consequences of pair housing in the WIFS model were explored in a pilot that only included rats housed in same-sex pairs (Mastroianni, 2011 unpublished data). It was found that pair housed males and females given wheel access had a four-day feeding suppression; both sexes had a shorter WIFS than seen in past studies with individually housed rats (Afonso and Eikelboom, 2003). It suggested that manipulating housing may have important social effects on feeding in the WIFS model.

Making changes in a rats' housing environment can have impacts on feeding levels. Male rats that have been chronically individually housed and then moved into pair housing have been shown to reduce their feeding for a total of three days compared to chronically individually housed or pair housed rats (O'Connor and Eikelboom, 2000). A follow-up experiment showed that the feeding suppression was not caused by the novelty of having an unfamiliar cage mate. It appeared to be caused by the housing change itself, the move from being chronically individually

housed to pair housed (Lopak and Eikelboom, 2000). This suggests that housing manipulations can change feeding and potentially also the WIFS.

Thesis aim

Wheel running, a rewarding behaviour, is highly influenced by social factors, including housing, and hormonal factors in female rats. It is evident that running has important consequences for energy balance. The consequences of running can become fatal if combined with food restriction in the ABA model but also appears problematic in the less severe WIFS model. Further work is needed to explore the outcomes of wheel running on energy balance in females. This work may have implications for paradoxical effects of human exercise, feeding, and body weight, hopefully shedding light on abnormalities like AN.

The present thesis explores several aspects of WIFS in female rats and compares them to the more commonly studied males. Sex differences in WIFS are examined, as well as the effects of variations in housing conditions upon wheel running, body weight and feeding in female rats. The WIFS procedure may lead to a better understanding of energy balance, eating disorders like AN, and excessive exercise. All of these factors are sexually dimorphic, yet work that compares females and males is limited. This thesis will look at variables that impact wheel running in females and running impacts on feeding and body weight, relative to males. The effects of housing manipulations (individual vs. pair housing) and social factors (presence or absence of males in colony room) in determining wheel running, body weight and food intake will be studied in females. Specifically, the effects of the presence or absence of males on estrous cycle synchrony and regularity in female rats will be explored by examining wheel running and feeding patterns.

Method

Subjects

176 female and male adult Sprague-Dawley rats from Charles River Laboratories in St. Constant, Quebec, Canada were housed in standard shoebox cages while adjusting to the laboratory for one week after arrival. Experiments were conducted in three replications: Replication 1 with 64 females (unisex housing), Replication 2 with 64 males (unisex housing) and Replication 3 with 48 females and males housed in the same room. Rats arrived weighing 200 to 225 grams with females being 57-70 days old and males 50-52 days old. Upon arrival, rats were randomly assigned to pair or individual housing in standard cages (24cm x 45cm x 19cm). A 12-hour light–dark cycle with lights on at 0800 or 0900 (in different replications) and a room temperature of 21 ± 2 degrees Celsius (humidity between 40 to 50%) was maintained for each replication. Rats were given *ad libitum* tap water and food (Rat Diet pellets with 3.11 kcal/gram of metabolizable energy; Harlan 8640, Madison, WI) access throughout the experiment. In all conditions, cages had hard wood beta chip bedding and a plastic bone for enrichment. 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

For the seven days after arrival, rats were in standard home cages with plastic tubes (8 cm in diameter and about 12 cm long) in addition to the bone. For the experimental phases, all rats were housed in similar sized cages with a locked or free-turning Nalgene running wheel (diameter 34.5 cm by 9.5 cm wide; approximately 1m in circumference).

General Procedure

After the rats' one week habituation to the laboratory environment, food for each cage was collected from the hoppers daily, weighed and then topped up to 160 grams. The body weight of each rat was also measured daily. For groups given unlocked wheel access, wheel turns were measured at a 10-minute interval, using the Vitalview 4.1 data collection system.

For Replication 1 and 2, the female and males were housed in unisex colony room conditions (refer to Figure 1 for a design summary of all replications). In Replication 3, females and males were housed together in the same colony room. In this replication, females were paired with females and males were always housed with males. Sexes were housed in different rows, but on the same cage rack.

After the 7 day habituation period, the rats were moved to new cages with locked wheels for a four day baseline period. During this period, food and body weight measurements were collected daily to provide a pre-running baseline. The 30 day experimental phase began after this baseline, during which food, body weight and wheel running were measured daily. Each replication had different rats. There were 32 pair housed rats in 16 cages for each replication. Replication 1 and 2 had 32 individually housed rats, while Replication 3 had 16. There were an equal number of rats in Replication 1 and 2 for each housing group. Replication 3 had more pairs than individually housed animals to allow for equal number of data points between housing groups for feeding and running. In each replication, half of the rats in each housing condition were given unlocked wheel access. The remaining individually and pair housed rats remained in cages with locked wheels, preventing the rats from running. Wheel rats were given thirty days of *ad libitum* wheel access, while the non-running rats served as controls with locked wheels. Thus, there were four groups in each replication: pair housed (PH) rats with wheel running access

(PW), PH rats in locked wheels (PNW), individually housed (IH) rats with wheel running access (IW) and IH rats in locked wheels (INW).

General Analysis

While it was possible to collect weight data daily for all rats individually, in the pair-housed conditions, there was only one data point for food consumption and wheel turns for the two animals. To parallel common reporting practice, feeding and running data of each pair of rats was divided by two to create an average data point for each pair – the equivalent of a single rat. All analysis for body weight was therefore done using individual rat data. Feeding and wheel running data was analyzed using the average value that reflected: the average of two rats for pair housed animals and individual animal data for all measures for the individually housed rats.

For each replication, data was analyzed in four day blocks: baseline (BASE) (Days -3 to 0), Wheel days (WD) 1-4 (Days 1 to 4), WD 5-8 (Days 5 to 8) and WD 27-30 (Days 27 to 30). BASE analysis permitted the examination of differences before wheel access. For wheel access specifically, analysis included the first eight days of the experiment (when major changes were expected) and the last four days of the experiment when all behaviours should have stabilized (Afonso and Eikelboom, 2003; Looy and Eikelboom,1989) . Analyses were done for Replication 1 (unisex housed females), 2 (unisex housed males) and 3 (females and males housed in the same colony room). Within each replication, the analysis first examined feeding, then wheel running. Note that a technical data collection problem occurred in Replication 2 and wheel running data for days 5 to 8 are missing (the male rats were still running) and thus for this replication WD 5-8 could not be analyzed. Finally, body weight was analyzed, where individual rat data was available.

Sex differences were expected as it is well known that females eat less and weigh less than males and also naturally run at higher levels (Wang, 1923; Eikelboom and Mills, 1988). Thus, the female and male rat data were analyzed separately. For each section, the analysis completed as a three-way mixed analysis of variance (ANOVA) for Housing (individual vs. pair housed) X Wheel (locked or free turning) X Days (4 days blocks).

In IBM SPSS Statistics 19 software, comparisons were made using this three-way ANOVA with significance set at $p<.05$. All repeated measures results that were reported as significant were also significant when using a Greenhouse–Geisser correction for potential violations of sphericity.

Additional Data Analysis of Females

In addition to exploring the evidence for WIFS and other feeding effects of running in female rats, this thesis explored how the presence or absence of males could affect the estrous-driven cycling of feeding and running. Effects of the estrous cycle could include the degree of synchrony among females and/or cyclic regularity. Estrous experiments often take vaginal smears and complete cytology to identify the period of estrus (Eckel et al., 2000). Vaginal smears were not collected in these experiments as the analysis was completed post hoc as estrous findings were unexpected. Estrus was instead identified indirectly using available measures: the peaks in wheel turns and dips in feeding, as these are known to be indicators of this cyclic stage (Wang, 1923; Eckel et al., 2000).

Synchrony and regularity were quantified using the daily wheel turn and food data obtained per cage/rat, although wheel running was used as the main indicator. When running stabilized (roughly day 15, halfway through the running period), the data for all females were re-

aligned so the high running and low feeding days (the estrus day) were assigned to day E0. Estrus was defined as the highest running or lowest feeding point in that particular four day period. In individual females, there was little difference for the day E0 as generated with individual feeding or running data suggesting this was an effective way of determining the day of estrous.

The number of realignments within a group needed to achieve synchrony on day E0 was quantified per group. A high number of realignments would be indicative of reduced synchrony. Day E0 is reflective of the highest synchrony scores. Data was realigned backwards and forwards from this point to track synchrony maintenance. For example, if all females were on a fixed four day estrous cycle, days –E4 and E4 would both be high running and low eating days. Note that all researchers, including the Animal Care staff that handled the animals, were female.

Results

Replication 1

Replication 1 included 64 females that were either individually or pair housed and half of the rats within each housing condition were given 30 days of wheel access. The females were housed without males in the room and were not exposed to the opposite sex at all. Daily food intake, wheel turns as applicable and body weight measures were taken.

Food intake

Figure 2 shows the food consumption over the experiment for the females. It is clear that wheel access had immediate effects on food consumption, initially by inducing a decrease in feeding for PW and IW rats compared to non-running controls. This was followed by a recovery from the WIFS for the running rats, which led to an increase in feeding compared to controls. Throughout this replication, housing condition had no systematic effect on feeding.

BASE: A three-way mixed ANOVA of the female rats feeding revealed a main effect of Housing, $F(1,44)=6.68$, $p=.01$. Figure 2 shows that during the baseline period, female pairs ate less than individually housed females. This difference in feeding was not evident in the males. Note, however, that this effect of Housing was small, as the mean for pair housed females was 22.20 g (SEM 0.26 grams) versus 23.59 g (SEM 0.28 grams) for individually housed females. There was no Wheel effect, indicating that running and control females did not have pre-existing differences in feeding.

WD 1-4: For females, the three -way mixed ANOVA for the initial wheel access days showed in a main effect of Wheel, $F(1,44)=36.43$, $p<.01$, evidence that running groups were

eating less than non-running controls (see Figure 2). There was no housing effect, highlighting that pairs and individually housed rats were eating similarly. There was a Days effect, $F(3,132)=26.72$, $p<.01$, as well as a Wheel X Days interaction, $F(3,132)=2.91$ $p=.04$. This interaction was due to the initial decrease in feeding, followed by increases in feeding that were more pronounced for the running rats than it was for the non-running rats.

WD 5-9: The three -way ANOVA during the second block of wheel access showed that there were no effects of the wheel or housing for the females. There was only a Days effect, $F(3, 132)=14.31$, $p<.01$, mostly reflecting increases in feeding seen in all groups. This suggests that the running rats had recovered from the WIFS and Figure 2 shows that this recovery occurred on roughly day 5.

WD 27-30: For the last block of running, the three-way ANOVA resulted in a main effect of Wheel, $F(1,44)=36.22$, $p<.01$. Figure 2 shows that, at this time, the running females were eating more than non-running controls. Additionally, there was a Days effect $F(3, 132)=6.00$, $p<.01$, and a Housing X Days $F(3, 132)=5.40$, $p<.01$, interaction. From Figure 2, it appears that food intake was changing over these days, but in an inconsistent manner across the four groups.

Wheel Turns

Figure 3 shows the wheel turn data for PW and IW rats for the female replication. A two-way mixed ANOVA (Housing X Days) was completed to compare these two groups for each of the three blocks. It was found that the IW rats had higher wheel turns than the PW rats for the entire wheel running period. Females overall showed some escalation in running over the first half of the running days and had more stable levels for the second half of the running period.

WD 1-4: A two-way analysis on wheel running for the first block showed only a significant main effect of Housing, $F(1,22)=12.76$, $p<.01$. As evident from Figure 3, the IW rats showed higher wheel turns counts than the PW rats over these four days.

WD 5-8: A two-way ANOVA for the second block of running showed a significant effect of Housing, $F(1,22)=12.39$, $p<.01$, and a Days effect, $F(1,22)=9.40$, $p<.01$. IW rats continued to show higher wheel turns than PW rats and there was an escalation in these counts for both groups. The escalation appears steeper for IW rats as is evident in Figure 3.

WD 27-30: Two-way analysis of the females for the last four days of the experiment showed that the Housing main effect was maintained, $F(1,22)=11.27$, $p<.01$. Figure 3 shows that the IW group continued to have higher wheel turns counts compared to the PW group. There was a Days effect, $F(3, 66) = 3.42$, $p=.02$, reflecting an upward increase in running occurring at over this running period.

Body Weight

Body weight measures were taken daily for each animal in Replication 1 before and during wheel running access. Pair vs. individually housed and running vs. non-running females were compared using a three-way mixed ANOVA (Housing X Wheel X Days). Figure 4 shows the average daily body weight for each group. The two running groups had a lower body weight than controls following wheel access. This difference was maintained throughout the experiment. There was no difference in body weight as a consequence of the housing condition.

BASE: A three-way ANOVA, revealed no main effects of Housing or Wheel and no interactions involving these effects, showing that there were no pre-existing group differences

across housing or wheel conditions. There was a Days effect, $F(3,180)=25.93$, $p<.01$, indicating that there was an increase in body weight across these four days.

WD 1-4: The three- way ANOVA of body weight for these days showed a significant main effect of Wheel, $F(1,60)=10.80$, $p<.01$, illustrating that the female running rats were gaining weight at a slower rate than the control rats (see Figure 4). There was a Days effect, $F(3,180)=96.20$, $p<.01$, as well as a within-subjects Wheel X Days, $F(3,180)=6.44$, $p<.01$ and Housing X Days , $F(3,180)=5.22$, $p<.01$ interaction. This suggests that body weight was changing differently for wheel and housing groups across these days. There was no main effect of housing, suggesting that the pair housed and individually housed rats were not significantly different in weight, despite showing different weight gains over these days.

WD 5-8: Three-way analysis of the females showed a between-subjects effect of Wheel, $F(1, 60)=16.83$, $p<.01$, for the second block of wheel access. There was main effect for Days, $F(3,180)=39.35$ $p<.01$ and a three way Days X Housing X Wheel interaction, $F(3, 180)=3.12$, $p=.03$. Looking at Figure 4, it shows that the two female running groups had a lower body weight than the controls.

WD 27-30: The females' three-way ANOVA showed a main effect of Wheel, $F(1, 60)=10.04$, $p<.01$ and a Days effect, $F(3, 180)=10.72$, $p<.01$. As with the previous blocks, there was no effect of housing. Figure 4 shows that the two housing groups with wheel access weighed less than the controls, even at the end of the experiment. All groups continued to gain weight. The PW and IW groups were similar in body weight, which was lower than that of PH and IH controls. There was no difference in the weight of the two control groups.

Replication 2

Replication 2 included 64 males that were either individually or pair housed; half of the rats within each housing condition were given 30 days of unlocked wheel access. The males were not housed with any females in the colony room. Daily food intake, wheel turns as applicable and body weight measures were taken.

Food intake

Figure 5 shows the food consumption over the experiment for all four groups of male rats. It is clear that wheel running had a profound effect on food consumption, by inducing an initial decrease in feeding compared to non-running controls. This initial WIFS was followed by a recovery when wheel running rats began to match the feeding level of controls. Housing condition did not have an effect on feeding. Note that, as expected, the males ate more than the females of Replication 1 (compare Figures 2 and 5).

BASE: The three-way mixed ANOVA of the baseline days revealed only a Days effect, $F(3,132)=25.87$, $p<.01$. Figure 5 shows that the males were generally increasing their feeding over these four days. There were no other significant main or interaction effects, highlighting that there were no pre-existing differences between housing conditions and running and non-running males prior to wheels being unlocked for half the rats.

WD1-4: The three-way mixed ANOVA for the first four days of unlocked wheel access for half the males found a significant main effect for Wheel, $F(1,44)=35.26$, $p<.01$, showing that running rats were eating less than non-running controls (see Figure 5). There was also a Wheel x Days interaction, $F(3,132)=4.75$, $p<.01$, reflecting a decrease over days in the reduction in

feeding in running rats and stable feeding for the non-running rats. There was no effect of housing on feeding.

WD5-9: The three-way ANOVA analysis for the second block with rats having unlocked wheel access showed main effects of Wheel, $F(1,44) = 6.52$, $p = .02$ and Days, $F(3,132) = 3.35$, $p = .02$. There was also a significant Wheel x Days, $F(3,132) = 3.52$, $p = .02$, and Housing x Days interaction, $F(3,132) = 5.02$, $p < .01$. Figure 5 shows that the running rats were eating less than the non-running controls, but that this difference was decreasing over these days. There was no main effect of housing, again indicating that pairs and individually housed animals did not differ for running or non-running groups.

WD27-30: The three-way ANOVA for the final four days of wheel access revealed only a within-subjects Days effect, $F(3, 132) = 5.86$, $p < .01$, reflecting slight fluctuations across days. There was no wheel effect and Figure 5 shows that the running and non-running rats were eating similar amounts. A housing effect was not evident, again showing that pairs and individually housed rats had similar feeding profiles.

Wheel Turns

Half of the rats within each housing condition were given 30 days of wheel access. The results of running can be seen in Figure 6 for the Replication 2 males. The graph shows that there was an effect of housing on wheel running (pair housed male rats ran more than individually housed rats) which was evident as long as the rats had access. Wheel running comparisons were done between pair housed (PW) and individually housed (IW) wheel rats for the first block and the last block (WD 1-4 and WD 27-30). The second block (WD 5-8) was not examined due to technical problems with wheel running collection at this time. Analysis was run as two-way

(Housing x Days) mixed ANOVA. As is evident in Figure 6, PW rats become high runners and showed higher wheel turns than IW rats. Looking at running patterns for a random few dark cycles, it appeared PW rats took few breaks and ran at near maximum levels for much of the night. The IW rats tended to take breaks throughout the night, running in typical bouts (Eikelboom and Mills, 1988).

WD1-4: A two-way ANOVA for the first four days of running revealed a main effect of Housing, $F(1,22)=5.06$, $p=.04$, as the PW rats ran more than the IW rats and a within-subjects Days effect, $F(1,22)=8.28$, $p<.01$, indicating that running was increasing over the four days (see Figure 6).

WD27-30: The two-way mixed ANOVA of the last block of running, days 27 to 30, revealed a similar pattern; a maintained main effect of Housing, $F(1,22)=16.66$, $p<.01$, illustrating that the PW rats continued to have higher wheel turns than the IW rats, and a Days effect, $F(3,66)=2.88$, $p=.04$. Figure 6 shows small but significant changes in running in the last block.

Body Weight

Body weight was measured daily for each animal throughout the experiment, from baseline to the last day of wheel running. As individual weights were obtainable, the analysis was completed with the raw data. The three-way mixed ANOVA analysis (Housing X Wheel X Days) was done for BASE, WD 1-4, 5-9 and 27-30. Average body weights for the males in the four groups are shown in Figure 6. It is evident that the PW and IW rats had a lower body weight than the two non-running control rats after wheel introduction, but that the PW rats had a much lower body weight than the IW rats.

BASE: During the four days of baseline, prior to wheel access, three-way analysis showed only a significant Days effect, $F(3,180)=262.95$, $p<.01$. There were no group differences between running and non-running or individual or pair housed groups in body weight prior to wheel access. The males showed an increase in body weight over these four days (see Figure 6).

WD1-4: During the first four days of wheel access, the body weight measures were compared using a three-way mixed ANOVA. There were significant main effects of Wheel, $F(3, 180)=7.85$, $p<.01$ and Days, $F(3,180)=349.91$, $p<.01$, and a significant Wheel x Days, $F(3, 180)=17.35$, $p<.01$ interaction. Figure 6 shows that the wheel group rats had a lower body weight than the non-running controls. At this point, there was no housing effect, showing that the PW and IW rats had a similar weight in both the running and non-running rats.

WD5-8: During the second block of wheel access, the three-way ANOVA results showed a main effect of Wheel, $F(3, 180)=12.97$, $p<.01$, and Days , $F(3,360)=427.53$, $p<.01$. Several within-subjects interactions were present; Housing x Days, $F(3,360)=4.24$, $p<.01$, Wheel x Days, $F(3,360)=4.80$, $p<.01$, and Housing x Days X Wheel $F(3, 180)=7.24$, $p<.01$. Figure 6 shows that the male wheel groups tended to have a lower weight than the controls, an effect which increased over days. It was also evident that the pair housed running rats showed the slowest weight gain, again an effect which increasingly evident over days and led to the significant triple interaction.

WD27-30: The group differences in body weight for the final block of running were examined. The three-way analysis produced a main effect for Wheel, $F(1,60)=20.52$, $p<.01$, a Days effect, $F(3, 180)=253.81$, $p<.01$, and a Housing X Wheel, $F(1,60)=7.94$, $p<.01$ and a three-

way interaction for Housing x Days X Wheel, $F(3, 180)=2.95$, $p=.03$ was present. As evident in Figure 6, PW and IW rats had a lower body weight than non-running controls during the last block of wheel running. The Housing X Wheel effect was driven by the PW rats having a much lower body weight than the other three groups (including the IW group) a difference that continued to increase even over the last four days of the experiment.

Replication 3

For Replication 3, data was analyzed in four blocks: BASE (Days -3 to 0), WD 1-4 (Days 1 to 4), WD 5-8 (Days 5 to 8); and WD 27-30 (Days 27 to 30) for food intake, wheel running and body weight. In this experiment, the females and males were in the same colony room simultaneously, but analyzed separately as a three-way mixed analysis of variance (ANOVA) for Housing X Wheel X Days. Comparison of females and males were not included because of the well-established sex difference.

Females

Replication 3 had 24 females that were either pair housed or individually housed in a colony room with 24 males. Half of the female animals within each condition were given 30 days of wheel access. To test the effect of the wheel, food intake, body weight and wheel running, analyses were done as three-way mixed ANOVA (Housing X Wheel X Days).

Food intake

Figure 8 shows the mean daily food intake for the females in Replication 3, before and after wheel introduction. There was an effect of wheel on the PW and IW rats; these two groups had a lower food intake compared to the non-running controls. Although the WIFS occurred in

these females, it recovered by day 5 and soon after PW and IW rats began to eat more than controls.

BASE: During baseline, a three-way ANOVA of the female feeding showed only a significant Days effect, $F(3,36)=7.49$, $p<.01$, reflecting day-to-day fluctuations in eating levels. There were no pre-existing group differences for these females.

WD 1-4: Three-way ANOVA for the first block of running showed a main effect of Wheel, $F(1,12)=12.96$, $p<.01$, and a Wheel X Days interaction, $F(3,36)=6.56$, $p<.01$. Figure 8 shows that the wheel running females ate less than the control rats but this difference decreased over days.

WD 5-8: The three-way ANOVA for the second block of running found no significant main effects or interactions. There was no effect of housing and the wheel effect was no longer present. Females in all groups were eating similar amount over these four days.

WD 27-30: The females' three-way mixed ANOVA for the last block of running showed only a main Wheel effect, $F(1,12)=9.25$ $p=.01$. Figure 8 shows that the IW and PW female rats were now eating more than the non-running controls.

Wheel Turns

Half of the females in each housing condition were given wheel access. The resulting wheel turn averages per day are shown in Figure 9. The PW rats had lower wheel turns than the IW rats. Females showed higher running than the males in this study. As can be seen in Figure 9, there was a pronounced 4-day cyclic effect in wheel running. On every fourth day, there was a

distinct peak in running that appears to be indicative of the night of estrus. It seemed that the females were peaking at the same time every four days, suggesting estrus synchrony and regularity were occurring.

WD 1-4: Two-way analysis of the females for the first block of running showed only a Days, $F(3,18)=6.99$, $p<.01$ effect, reflecting the increase in running that was occurring (Figure 9). These results suggest there were no housing differences in running for the IW and PW rats.

WD 5-8: The two way ANOVA for these days revealed a Housing effect that trended towards significance, $F(1,6)=5.88$, $p=.052$. There was a significant Days effect, $F(3,18)=11.70$, $p<.01$ and a Days X Housing, $F(3,18)=3.53$, $p=.04$ interaction. This suggests that differences between IW and PW were emerging, as running was increasing over days (see Figure 9).

WD 27-30: Two-way ANOVA for the last block of running for the females showed only a significant Days effect, $F(3,18)=8.44$ $p<.01$. There were no significant housing differences.

Body Weight

Body weight measures were taken for each individual animal throughout the experiment. Figure 10 shows the mean daily body weight for the females across the experiment. Running animals had a lower body weight that was lower than non-running rats. This effect of wheel was evident throughout the experiment. No weight differences due to the housing manipulation were evident.

BASE: The three-way ANOVA showed only a significant Days effect, $F(3,60)=22.31$, $p<.01$, reflective of the increase in weight over days.

WD 1-4: The females' three-way ANOVA for these days showed a main effect of Wheel, $F(1,20)=5.15$, $p=.04$, Days, $F(3,60)=9.15$, $p<.01$, and a Days X Housing X Wheel interaction, $F(3,60)=3.10$, $p=.03$. Figure 10 shows that the PW and IW females weighed less than the control rats. Weight gain over these days was inconsistent over the rats in the four groups.

WD 5-8: The three-way ANOVA for the females in the second block of running showed only a significant Wheel effect, $F(1,20)=5.52$, $p=.03$. Figure 10 shows that the female running rats had a lower body weight than the controls. There was no Housing effect, meaning that PW and IW rats were equally affected by the wheel and the control housing conditions did not differ.

WD 27-30: The three way ANOVA for the females at the end of the experiment revealed a Wheel effect, $F(1,20)=5.41$, $p=.03$; the wheel rats continued to weigh less than controls and a repeated Days effect, $F(3, 60)=3.17$, $p=.03$, reflecting a daily increase in body weight for each group.

Males

Food intake

Figure 11 shows the average daily food intake for each of the 4 male groups in Replication 3. It is clear that the wheel access had an effect on feeding for the PW and IW groups. The wheel groups initially ate less than the non-running groups, indicative of a WIFS that lasted close to 7 days before the running rats increased their consumption above that of the control rats. There appeared to be no effect of housing on food intake.

BASE: Similarly to Replication 1 and 2, food intake measures were daily taken for all animals for a baseline period of four days prior to wheel access. The three-way ANOVA showed

only a day-to-day variance in eating, as there was a significant Days effect, $F(3, 36)=20.34$, $p<.01$. The male rats in the four groups were not different prior to wheel access.

WD 1-4: Wheel access began on WD 1 to 4 for half of the male rats. Three-way ANOVA showed a significant main effect of Wheel, $F(1,12)=12.36$, $p<.01$, Days, $F(3,36)=5.78$, $p<.01$ and a Days X Wheel, $F(3,36)=10.05$, $p<.01$ interaction. The differences seen in Figure 11 suggest that running group rats were reducing their feeding relative to non-running rats. The difference in feeding decreased across days resulting in the significant interaction. There was no effect of the housing manipulation on feeding.

WD 5-8: During the second block of wheel access, the three-way ANOVA showed that there was only a significant Days effect, $F(3,36)=4.55$, $p<.01$ indicating changes feeding over days but no effect of wheel or housing on feeding. Figure 11 demonstrates that the WIFS was no longer evident for the running group rats over these days.

WD 27-30: During the last four days of wheel running, three-way ANOVA indicated an overall Wheel effect, $F(1,12)=16.92$, $p<.01$ and a Days effect, $F(3,36)=3.74$, $p<.02$. From Figure 11, it is evident that the PW and IW group rats were now eating significantly more than the equivalent non-running rats but it appeared that feeding varied over days.

Wheel Turns

Half of the Replication 3 rats were given 30 days of wheel access. Comparisons were made between housing groups with a two-way mixed ANOVA analysis (Housing X Days) for three blocks: WD 1-4, 5-8 and 27-29. Figure 12 shows the wheel running data for the males over

the 30 days. There were no significant differences between PW and IW; however, the group sizes of 4 rats may have masked potential differences.

WD 1-4: The two-way ANOVA for the first four days of wheel running showed no significant main effects or interaction. Figure 12 shows that were no housing differences between PW and IW and there was no real increase in running over these days.

WD 5-8: The two-way analysis of the second block of running, days 5 to 9, showed only a significant Days effect, $F(3,18)=10.41$, $p<.01$, reflecting the increase in running seen in Figure 12. There were no significant differences between housing groups.

WD 27-30: The ANOVA for the last block of running, days 27 to 30, showed only a significant Days effect, $F(3,18)=4.50$ $p=.02$. This suggests that, after 26 days of wheel running, there were still day to day differences in running. However, there were no housing differences between the PW and IW groups.

Body Weight

Daily body weight measures were taken for each animal throughout the experiment, from baseline through the 30 days of wheel access. Analysis was done for each block overall as a three-way ANOVA (Housing X Wheel X Days). Figure 13 shows the mean body weight for the males across the experiment. Running animals gained weight more slowly relative to control males after wheel introduction. Within these males, there was never a distinct housing effect.

BASE: Body weight analysis was done for during baseline, for four days prior to wheel access for all animals. The three-way analysis showed a only significant Days effect,

$F(3,60)=586.18$, $p<.01$. These findings suggest that there were no pre-existing group differences for housing or wheel conditions, just a steady weight gain for all rats.

WD 1-4: Body weight three-way ANOVA comparisons were made for the first four days of wheel access. There was only a Days X Wheel effect, $F(3, 60)=3.34$, $p=.03$, suggesting that differences may be emerging between the wheel and non-wheel groups of rats that increases across these four days.

WD 5-8: Three-way ANOVA for the second block of running demonstrated a Days effect, $F(3,60)=51.07$, $p<.01$, and a Days X Wheel interaction, $F(3,60)=2.83$, $p=.05$. The running rats were not gaining weight as fast as the non-running rats. Although in Figure 13, there seems to be a overall difference between running and non-running rats, the Wheel main effect was not significant, but there was a trend, $F(1,20)=3.87$, $p=.06$.

WD 27-30: Three-way ANOVA of the last block of running shows a Days effect, $F(3, 60)=27.71$, $p<.01$, reflecting the growth in body weight over days for all groups. The Wheel effect was also significant, $F(1,20)=20.28$, $p<.01$, showing that the running rats weighed less than controls. There was no significant effect of Housing.

Summary of Findings

In this section, the results from all three replications will be brought together for a single review so that all findings may be compared and contrasted. As appropriate, I will also include some of the immediately relevant literature in this overview.

Food: Replication 1 results show (see Figure 2) that, after wheel access, all wheel running females experienced a WIFS and reduced their feeding amounts compared to non-

running rats. Over the first 24 hours of wheel access, the PW rats decreased their feeding by 22% (5 grams) compared to PNW rats; IW rats decreased their feeding by 26% (6 grams) compared to INW rats. The decrease from BASE to the WD 1 for IW rats was a 24% decrease (5 grams), while it was a 25% decrease (6 grams) for the pairs. The WIFS lasted only four days for the female PW and IW groups. Over the fifth to eighth days of wheel access, both female wheel groups ate amounts similar to the non-running groups. Gradually after the short WIFS, the female IW and PW groups began to eat more than the controls, an effect clearly evident in the last four days of this replication. The elevation suggests that the rats were compensating for the loss of calories caused by the wheel. During the baseline period, the pair housed females were eating less than the individually housed rats, but this difference was not evident again after this block. Thus, for the females (like with the males), the housing condition did not impact the WIFS as the PW and IW did not have any significant differences. The female wheel groups had a shorter WIFS compared to the males in Replication 2 and to past studies done with males (Afonso and Eikelboom, 2003).

The analysis of Replication 2 males showed a significant effect of wheel on feeding for the first and second block of running, days 1 to 8. Figure 5 suggests that it lasted roughly 7 days before food consumption and returned to levels of the non-running males. Over the first 24 hours of wheel access, the PW rats decreased their feeding by 19% (7 grams) compared to PNW rats; IW rats decreased their feeding by 25% (9 grams) compared to INW rats. The decrease from BASE to the WD 1 for IW rats was a 20% decrease (7 grams), while it was an 11% decrease (4 grams) for the PW rats. Observing the last four days of wheel running in Figure 5, the running males did not show any elevation in feeding relative to non-running controls. Thus, IW and PW males did not demonstrate the elevated eating seen in the female wheel rats upon WIFS recovery

in both replications. Pair housing failed to have an effect on the WIFS as both male wheel groups showed a similar suppression both in duration and severity.

In Replication 3, females and males experienced a similar initial drop in feeding, relative to their respective non-running controls. For the females : over the first 24 hours of wheel access, the PW rats decreased their feeding by 46% (10 grams) compared to PNW rats; IW rats decreased their feeding by 30% (7 grams) compared to INW rats. The decrease from BASE to the WD 1 for IW rats was a 21% decrease (5 grams), while it was a 41% decrease (8 grams) for PW rats.

For the males : over the first 24 hours of wheel access, PW rats decreased feeding by 33% (10 grams) compared to PNW; IW rats decreased feeding by 34% (10 grams) compared to INW. The decrease from BASE to WD 1 for IW rats was a 38% decrease (11 grams), while it was a 32% decrease (10 grams) for PW rats.

Although comparisons of the first two replications showed a sex differences in the WIFS duration, with females having a shorter duration, both sexes had a short duration WIFS in Replication 3. The females and males both recovered from the WIFS by the fifth day as there was no wheel effect or interaction in the analysis of days 5 to 8. Like in the two previous replications, there was no effect on the housing on the feeding as pair or individual housing did not affect the WIFS. Both females and males recovered from the WIFS quickly and, as evident in the final four days of the experiment, wheel running rats were eating more than controls. Unlike the first replication, the males appear to have compensated for the running by increasing feeding relative to controls. The females reliably compensated for the cost of running, while this compensation only occurred in one of these replications for the males.

Wheel Running: Unlike the effect of the housing manipulation on feeding in both males and females, the pair housing had complex effects on running. For females, the pair housed rats showed lower running amounts in both replications than individually housed females (see Figures 3 and 9). It appears that this is an artifact of females running together in the wheel. For males, the situation was less clear in that the running patterns were dissimilar in Replication 1 and 3.

Replication 1 found that, after the initial increase in running, female IW rats averaged just over 10,000 wheel turns across the 30 days of wheel access (see Figure 3). This average is typical of the female running patterns found in past studies (Eckel et al., 2000). Over the same period, the PW rats averaged at 4500 wheel turns; less than half the amount run by the IW rats. However, it is highly likely that the female pairs were sharing the wheel as this was often observed anecdotally when handling the rats. If female pairs ran together, it is possible that a significant amount of the wheel turns were reduced in half since two animals were running simultaneously. Had the females shared the wheel 100 percent of the time, their average wheel turns would actually amount to 9000 wheel turns, which is what is seen in the individually housed rats. Further analysis, such as videotaping the behaviour at night would be required to actually determine the actual wheel sharing patterns.

As is evident in Figure 6, Replication 2 male IW rats ran at typical levels for adult males. They peaked close to 5,000 wheel turns in a 24 hour period, as evident in previous running studies (Afonso and Eikelboom, Eikelboom and Mills, 1988). After an escalation in running over the first number of days of wheel access, males tend to peak and then remain at that level for the remainder of wheel access. IW males in Replication 2 and 3 began running at low levels (roughly 1000 wheel turns) and began increasing steadily, reached the peak and then stabilized.

The PW rats in Replication 2showed running levels that were higher than typically seen. All PW rats were high runners throughout the experiment, peaking at 9,000 wheel turns (18,000 wheel turns for the pair). In fact, these PW rats ran at similar levels to female individually housed rats in Replication 1 (see Figure 3). It appears that placing rats in pairs with access to only one wheel resulted in very high and continuous levels of running. Running over the night did not show the breaks in running typically seen in other studies. The high running in the PW rats also resulted in a large body weight difference, with these rats showing a much lower weight than the males in the IW, PNW and INW groups. Body weight between each pair did not show large differences, suggesting that both rats in the pair were running significant amounts. Given the lack of compensation in feeding, this weight difference suggests that the high levels of running are real. It was hypothesized that the males in the pair housing condition were competing for the wheel as it was a shared resource. However, the males in Replication 3 failed to demonstrate this effect as PW and IW rats ran at similar levels. Sharing was not seen but the pairs did not run at atypical high levels. It should be noted that, in an unreported study of pair housed males in a colony room with no females present, the effect was also not evident (Mastroianni, 2012 unpublished data).

Replication 3 wheel running data showed that the females ran more than the males throughout the experiment, a finding consistent with past work (Eikelboom and Mills, 1988). Interestingly, in this replication, neither sex showed a difference between IW and PW rats. Although females were trending towards a housing difference as IW appeared higher than PW rats (Figure 9).

Replication 3 females showed a different running pattern than seen in the males. The females had a higher activity level than males. IW and PW females both showed an escalation in running, but the day-to-day pattern was less stable (see Figure 9). It is notable in Figure 14 that a

highly distinct 4-day cycle was occurring in the wheel running, showing peaks and dips within each four days. The IW peaks in running would occur every forth day, hitting roughly 17,000 wheel turns, while the dips were roughly 10,000 wheel turns. The peaks and dips were less pronounced in the PW, but this may be due to the shared wheel behaviour. The pattern suggested that the females' running was highly driven by the estrous cycle. IW rats averaged over 13,000 wheel turns, while the PW group rats averaged 8000 wheel turns. As in Replication 1 and 2, females were anecdotally running in the wheel together, while the males were not. The PW rats again showed fewer wheel turns on average compared to the IW rats; however the difference was not significant. This may have been due to the small group sizes (4 wheels in each condition).

As is evident from Figure 12, the males showed a steady yet modest escalation in running over the experiment, with both housing groups showing similar exercise patterns. Both male groups peaked at roughly 6000 wheel turns about half way through wheel access, with PW averaging at roughly 5000 wheel turns and IW averaging a little higher at 6000.

Body weight: As would be expected for all replications, the females weighed less than the males. In each replication, all rats introduced to unlocked wheels had a small drop in body weight immediately after wheel access, relative to non-running controls (see Figures 4, 7, 10 and 13). After this initial effect, all wheel rats showed increases in their body weight at a similar rate to the non-running rats. However, wheel rats remained at a lower body weight than control rats throughout the experiment.

In Replication 2, the male PW rats gained weight at a much slower rate, compared to the other three groups. By the last day of the experiment, these rats weighed close to 50 grams less than IW rats. The decreased body weight differences in the PW rats reflect the unexpectedly high level of running that was seen in this group.

For Replication 3 males, wheel access did not show the expected effect on body weight for the first 8 days of running but the expected difference did emerge in the last block. It is possible that the small group size masked the initial effect or the wheel males were slower to experience the body weight effects because of their relatively low levels of running.

Estrous cycle for Replication 3 and 1

Post hoc, it was noticed that the individually housed female rats (Replication 3) that were housed with males in the room displayed and maintained a marked synchronous and regular cycle and the pair housed rats also displayed some evidence for regularity (see Figures 14 and 15). The wheel running pattern shows marked dips and peaks in feeding every four days, reflective of the night of estrus (peaks) and diestrus (dips). For the most part, these cyclic patterns in running matched similar but reversed patterns seen in their feeding. Replication 1 showed hints of this estrous-induced pattern of running and feeding (see Figures 16 and 17), but the synchrony and regularity was less pronounced.

Replication 3

Figures 14 and 15 show the wheel running and food intake data for IW and PW females of Replication 3. IH rats that were housed with males in the room displayed a marked synchronous and regular cycle, while the pair housed rats displayed a similar cyclic regularity; the peaks and dips were not as extreme and more realignment was necessary. IW females had a very regular 4-day estrous cycle, with estrus occurring synchronously. Figure 14 shows very pronounced dips in feeding when peaks in wheel running occurred. For IW females, 1 of 4 cages needed to be realigned in order to synchronize estrus. The degree of realignment required for pair

housed rats was 2 of 4 cages. Only 1 day's realignment was needed for the 4 cages in the IW rats, whereas there needed to be 2 cages shifted one day each for the 4 cages of the PW rats.

Replication 1

Figure 15 and 16 display the wheel running and food intake data for IW and PW animals in Replication 1, who were housed without males. The females data was realigned so that day E0 was a peak day of running used to define estrous. Due to a few unreliable wheel data collection days during the period when running had stabilized, 3 IW rats and 1 PW rat were removed from estrous analysis. (Note that, in regards to the general analysis for females, no animals were removed; WD 1 to 8 and 27 to 30 did not show significant wheel running data collection problems). IW and PW female rats did not appear to display marked estrous cycle synchrony, relative to rats housed with males in the room in Replication 3. As seen in the Figures 15 and 16, the changes at estrus were less pronounced (even less so in pair rats) with larger loss of synchrony. Of the IW females, 69% of cages needed to be realigned in order to be on estrus (day E0). The degree of realignment required for pair housed rats was 57%. The average number of days that needed to be realigned per cage was 0.85 for individually housed rats, whereas it was 1 for pair housed rats. Pair housed females housed with males (Replication 3) seemed to show more regularity and synchrony than female rats pair housed without males, but the individually females housed with males clearly showed the most synchrony and regularity.

Discussion

With a focus on female rats, this thesis explored factors that control wheel running, and looked at its immediate and delayed effects on body weight and food intake. Male rats were studied as well to allow for sex comparisons and to permit a comparison to the largely male literature. Housing manipulations, individual or pair housing, allowed for examining the effect of social interaction on running and the WIFS. Finally, the consequences of male presence or absence on the female behaviours were explored by housing the females in colony rooms with or without male rats.

Wheel access caused a temporary reduction in feeding and a lasting reduced body weight in both females and males, compared to non-running controls. This phenomenon is known as the WIFS (Afonso and Eikelboom, 2003). WIFS has been suggested as an animal model of AN, as this disorder often has an exercise-eating interaction (Davis, 1997; Beumont et al., 1994). Both males and females were affected by the introduction of an unlocked running wheel and reduced their feeding short-term, maintaining a lower body weight throughout wheel access. It was found that pair housing failed to have an effect on the WIFS. However, housing and sex differences emerged in wheel running, where females ran in the wheel together and males did not. This thesis confirmed that WIFS occurs in females, but that the duration may often be shorter than the WIFS seen in males. The initial drop in feeding seen with the WIFS was similar for females and males in both replications. After recovery from the WIFS, females with running access (in both the second and third replication) quickly came to eat more than non-running animals. Males only showed an increase in feeding in the third replication but not in the second replication. Thus, the later increase in feeding was constantly seen in females but was inconsistently evident in males. Previous studies have been mixed as males have been shown to either match the feeding levels of

controls or significantly increase it as a temporary hyperphagia (Premack and Premack, 1963; Looy and Eikelboom, 1988; Afonso and Eikelboom, 2003). Females, on the other hand, seem to consistently recover and then begin to eat at higher levels than the controls both in this thesis and in previous work (Dalton-Jez, 2006; Afonso, 2000). For the males, it is not clear if the compensation is slower in occurring or sometimes does not occur at all. It is unclear if this sex difference is driven by a quicker compensation in the females compared to the males for the calorie increase required after wheel running. Another possibility is that the increase in feeding is controlled by the female ovarian hormones or perhaps another unknown mechanism is driving the sex differences. This is evidence that females are better than males at regulating during periods of energy imbalance. Note that, because females weigh less and gain body weight at a slower rate even without wheel access, they tend to eat less than males; leading to the possibility that they have to compensate for weight challenges more quickly. WIFS sex comparisons become somewhat challenging as we are comparing animals with pre-existing feeding and body weight growth differences, despite initially ordering the rats by weight so that they started out with equal weights.

In humans, 36% of AN patients transition into bulimia nervosa, an eating disorder characterized by binge eating followed by purging, often within 5 years of illness (Tozzi et al., 2005). It is possible that this is reflective of the hyperphagia seen after WIFS recovery. In other words, perhaps AN patients engage in energy balance regulation by increasing consumption, but pathology causes them to purge the food to stay thin. However, though some over-eating consistently occurs, the size of the feeding difference between wheel rats and controls do not always reach significance (for females in Replication 3, this was perhaps due to small sample size). It is possible that the transition from AN to bulimia may not relate, however, as exposing

female and male rats in adolescence to the ABA procedure does not result in "binge-eating" in adulthood when given short daily access to a palatable food for 2 h/day and unlimited access to chow (Cai et al., 2008).

The WIFS for males in past work is typically pronounced and lasts 7-12 days (Afonso and Eikelboom, 2003). Some variance in this range has been seen in the past, so it is not entirely atypical to see a shorter suppression in the males. The females in this thesis showed a shorter WIFS duration than what is typically seen in males. It was only when males showed a short duration of the WIFS that the sexes appeared to be similar. Some studies that provided female rats with wheel access have concluded that exercise has either no effect on food intake or has only a small stimulatory effect, as well as having no effect on body weight. (Anantharaman-Barr and Decombaz, 1989; Eckel and Moore, 2004; Eckel et al., 2000). These studies took weekly body weight and food intake measures, missing the initial food intake reduction and differences in body weight. They did not look at daily changes in weight and feeding and looked at overall averages instead. Studies that stated this used young rats that likely had not yet experienced puberty. Studies that provided post puberty adult rats (50 days) with wheel access and *ad libitum* food that females reduced their intake (Tokuyama and Okuda, 1982). This thesis further demonstrates is that the WIFS does occur in females and the food intake initial drop after wheel access and severity of the reduction is comparable to that of male wheel rats but typically of shorter duration.

Despite feeding differences within the WIFS, both females and males with running wheel access maintained a lower body weight than non-running controls after wheel introduction, a difference that was maintained for the duration of the experiment. Only in Replication 2 males

was there a housing-induced difference in body weight, possibly caused by the unusually high running seen in the pair housed male rats.

Pair housing, thought to provide a closer match to what happens in the natural environment and so would potentially reduce stress for the rats, was hypothesized to be a manipulation that might mitigate the WIFS. In all the replications of this thesis, the housing manipulations failed to change the WIFS. This is despite previous work that shows that social support, which in rats can mean group housing, can lead to improved health outcomes (Baldwin et al., 1995). The WIFS was always the same in pair and individually housed wheel male and female rats; wheel running induced similar reductions in feeding and body weight for all replications. This finding was consistent through all studies. It is possible there could be success by housing more rats together (other studies have used 4 or 5), as larger groups can result in more reduced stress levels (within space restraints; crowding can have the opposite result) (Martí et al., 1994; Vallès et al., 2012). Technology to measure individual wheel and food intake would be required. Another possibility for why the housing manipulation was not effective in reducing the WIFS was because it was chronic. Perhaps if animals were originally individually housed and then some animals were moved into pair housing several days before or even after wheel access, different housing results could be seen.

The housing manipulation had a complicated effect on wheel running. Pair housed females had lower wheel turn counts than individually housed females. It appeared that this difference may have been an artifact of both females running in the wheel together. Males were never observed running in the wheel simultaneously and showed a pattern that was different in the two replications. Pair housed males had higher wheel turns than individually housed rats in Replication 2 but the two housing conditions showed equal running in Replication 3. Further

observation data for the females (and males) is required. It is possible that the females shared the wheel (at least some of the time), while the males did not appear to run together due to relative differences in size. Males are naturally larger in size and heavier in weight. It is possible that it is challenging for male pairs to run together, while female pairs can fit in the wheel together much easier. Should a size reason be ruled out, it is possible that females and males behave differently when working with a shared reward. Males may demonstrate increased competition over limited resources compared to females as they are less willing to form social bonds with the same sex (Beatty, 1979). It seems plausible as females continued to run together even towards the end of the experiment, when they were exceeding 350 grams. Note that males achieved this weight towards the beginning of the experiment. This would be difficult to explore as, if one were to attempt to match the size of the sexes, age or developmental issues would arise. Including males that are as light as adult females would likely have to run into the lifespan prior to puberty. As mentioned, the WIFS does not occur prior to puberty, nor do true sex differences in wheel running (Dalton-Jez, 2006; Afonso, 2000). In order to uncover potentially interesting social differences between males and females when sharing a rewarding resource (wheel), further investigation is required. Another problem with using lighter males and heavier females of the same age is the risk that these animals are "different" than the rest of the subject population (for example, the male may have less testosterone or high levels of stress). One potential way to get at this question is to have a larger wheel within a larger cage so that the wheel is still a shared resource, but the males could both fit inside in order to run together.

Females and males were housed in separate unisex rooms in the first two replications and in the same room in Replication 3. This allowed for testing the effects that male presence can have on estrous synchrony and cyclic regularity in females by measuring changes in wheel

running, which reliably coincide with the estrous cycle. Pair housing permitted the potential exploration of close proximity on the estrous cycle. It seemed that the presence of males in Replication 3 increased female synchrony and regularity as seen in wheel running patterns, relative the females in Replication 1 that were not exposed to males. It proved difficult to disentangle the effects of close proximity in pair housed females as they were sharing one wheel and individual running was not available. Nonetheless, less evidence for synchrony and regularity were seen in pairs compared to individually housed females even in the presence of males in Replication 3. The absence of males was evidenced in females by irregular and random estrous cycles. The peaks in running and the dips in feeding were less pronounced in the PW rats compared to IW rats. Replication 3 PW rats with males present had more evidence of synchrony and regularity than Replication 1 PW rats, but in both cases less than that seen in the parallel IW females. The running synchrony in the presence of males suggests that the male rats may have induced a Whitten effect in female rats (Whitten, 1956). The absence of males may have conversely resulted in a Lee-Boot effect – longer and more irregular cycles (Lee and Boot, 1956). A limitation of this estrous finding is that vaginal smears were not obtained to verify the point in the estrous cycle. Additionally, a larger sample size for experiment 2 would have been beneficial in validating the result.

An important overall limitation of this study, as well as other work with the ABA or WIFS model, is the abnormal conditions of the laboratory. Laboratory animals are housed in small cages with minimal enrichment, which is not indicative of a natural environment. Especially with individual housing, rats only have access to food without any other form of reward or stimulation. As a result, most rats become obese and are not ‘normal’; Boggiano et al. (2008) suggest that stressed animals do not make good research subjects for this reason. When

performing experimental manipulations, like providing rats with wheel access, it is not clear if any effects are due to the manipulation or to the abnormal control conditions/subjects.

Exercise has important effects on energy balance and can sometimes result in paradoxical reduced appetite, which can be seen in rats and humans of both sexes. Appetite suppression induced by exercise is seemingly mediated by increased catecholamines (Oscai et al., 1997), likely placing the body into fight-or-flight mode, and the reduced food intake can in turn increase drive for physical activity (Beumont et al., 1994). In order to prevent pathology from occurring, such as that seen in AN patients, regulation of energy balance is required through an increase in food intake as exercise increases.

In humans, females have a higher incidence of AN than males do (Davis, 1997). This somewhat contradicts the sex differences seen in WIFS but parallels it in other ways. Given that females run more than males (Wang, 1923), they are in fact generating a larger negative energy balance. This would suggest that females should not reduce their feeding as much as males. Despite this, females demonstrate a similar degree of WIFS as males. This suggests the females are more vulnerable because they are at a greater negative energy balance than males. However, females recover from the feeding suppression quickly, while males usually (but not always) show a longer feeding suppression. Females consistently increase food intake in a short period after recovery; males do not always increase their feeding after recovery. In that sense, female rats in WIFS work are difference from female AN patients, as they show enhanced ability to regulate their energy balance (Anantharaman-Barr, H. G., and Decombaz, 1989). These sex differences are likely driven by gonadal hormones (Beatty, 1979) but to date no one has looked at WIFS in rats without their gonads using hormone replacement. Work is also needed to assess energy expenditure by measuring heat production within the WIFS model, in order to identify

sex differences that can contribute to feeding and body weight outcomes (Anantharaman-Barr, H. G., and Decombaz, 1989).

This work suggests that exercise affects female differently than it does males. With prolonged physical activity (9 weeks), Cortright et al. (1997) found that male rats showed a reduced fat, protein and body mass compared to sedentary controls, while running females were similar to sedentary controls in this regard. This is reflective of work in humans demonstrating that males are more susceptible to fat loss than females when undergoing an endurance exercise program (Bjorntorp, 1989). Females may possibly be less affected by and less sensitive to exercise than males, suggesting that females would better survive in periods of food scarcity; males would do better in times of excessive food availability (what is seen in Western societies today) (Cortright et al., 1997). This may provide some basis for why women are more obese globally than males (World Health Organization, 2008). However, this does not explain why AN occurs more frequently in females. It is possible that social pressures to be thin (which are present for females and less for males in Western cultures) and genetics are the cause for this sex difference (Beumont et al.,1994) Work should continue to examine the biology behind AN in patients that exercise excessively to see if a biological factor is responsible for the deficiency in adapting to increased activity. When exploring animal models of AN, females and males should be included to understand the sex differences in energy balance following exercise.

In conclusion, this study showed that WIFS is a viable model for AN as introducing wheel running to rats induces high levels of physical activity and reduced intake initially, similar to patients with AN that exercise excessively while self-starving. WIFS allows for tracking appetitive behaviour post recovery from self-starvation, which, with more work, could be important for tracking progress in AN treatment programs. Continuing to include females in

WIFS work in order to make extensions towards AN is an appropriate path for future studies, given the gender difference in this disorder. Female rats show similar initial reductions in feeding as males when given wheel access and demonstrate a suppression that is significant, although with a shorter duration than typically seen in males. Future work is required to disentangle the mechanisms that drive WIFS sex differences in females and males. Working with different female rodent species would be a good starting point, to see if sex differences are specific to rats or in male and female rats with a loss of their gonads (and then given hormone replacement) . AN is a deadly disorder and investigation with female rodents may contribute to better understanding of the factors behind its etiology and maintenance, as well as potential prevention and treatment.

References

Afonso, V. (2000). *Wheel running, feeding and body weight in adult male rats: Generalizations to adult females and younger animals.* (Master's thesis). Retrieved from Scholars Common @ Laurier.

Afonso, V. & Eikelboom, R. (2003). Relationship between wheel running, feeding, drinking, and body weight in male rats. *Physiology & Behavior*, *80*, 19–26.

American Psychiatric Association. (2000). Diagnostic and statistical manual of mental disorders (4th ed., text rev.). doi:10.1176/appi.books.9780890423349.

Anantharaman-Barr, H.G., & Decombaz, J. (1989). The effect of wheel running and the estrous cycle on energy expenditure in female rats. *Physiology & Behavior*, *46*, 259–63.

Baldwin, D.R., Wilcox, Z.C., & Baylosis, R.C. (1995). Impact of differential housing on humoral immunity following exposure to an acute stressor in rats. *Physiology & Behavior*, *57*, 649–53.

Barlow, D.H., Durand, V.M., & Stewart, S.H. (2012). Major Types of Eating Disorders In *Abnormal Psychology: An Integrative Approach* (pp.263-265). Belmont, California: Wadsworth Publishing.

Beatty, W.W. (1979). Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organizational and activational influences. *Hormones and behavior, 12*, 112-63.

Beumont P.J., Arthur B., Russell J.D., Touyz, S.W. (1994). Excessive physical activity in dieting disorder patients: proposals for a supervised exercise program. *International Journal of Eating Disorders, 15*, 21-36.

Beumont, C.C., Beumont, P.J. & Touyz, S.W. (1996). The problem of excessive activity in patients with anorexia nervosa. In W.F Epling & W.D Pierce (Eds.), *Activity Anorexia: Theory, research and treatment* (pp. 189-198). Mahwah, NJ: Erlbaum.

Boggiano, M.M., Cavigelli, S. A.; Dorsey, J.R.; Kelley, C.E.P.; Ragan, C.M.; et al. (2008), Effect of a cage divider permitting social stimuli on stress and food intake in rats. *Physiology & Behavior, 95*, 222-228.

Boakes, R.A., Mills, K.J. & Single, J.P. (1999). Sex Differences in the Relationship Between Activity and Weight Loss in the Rat. *Behavioral Neuroscience, 113*, 1080-1089.

Bulik, C.M., Sullivan, P.F., Wade, T.D. & Kendler, K.S. (2000). Twin studies of eating disorders: a review. *International Journal of Eating Disorders, 27,* 1-20.

Canada. Public Health Agency of Canada (2005). A report on mental illnesses in Canada (Chapter 6). Retrieved from Public Health Agency of Canada website: http://www.phac-aspc.gc.ca/publicat/miic-mmac/chap_6-eng.php.

Cai, W., Bocarsly, M.E., Arner, C.N., Walsh, B.T., Foltin, R.W., Hoebel, B.G., & Barbarich-Marsteller, N.C. (2008). Activity-based anorexia during adolescence does not promote binge eating during adulthood in female rats. *The International Journal of Eating Disorders*, *41*, 681–685.

Clarke, T.K., Weiss, A.R.D. & Berrettini. (2012). The Genetics of Anorexia Nervosa. *Clinical Pharmacology & Therapeutics, 91,* 181-188.

Collier, G., Hirsch, E., Levitsky, D. & Leshner, A.I. (1975). Effort as a dimension of spontaneous activity in rats. *Journal of Comparative and Physiological Psychology*, *88,* 89–96.

Cortright, R.N., Chandler, M.P., Lemon, P.W., & DiCarlo, S.E. (1997). Daily exercise reduces fat, protein and body mass in male but not female rats. *Physiology & Behavior*, *62*, 105–11.

Cooper, K. & Haynes, N.B. (1967). Modification of the oestrus cycle of the under-fed rat associated with the presence of the male. *Journal of reproductive fertility*, 317–320.

Dalla, C., Antoniou, K., Drossopoulou, G., Xagoraris, M., Kokras, N., & Sfikakis, A. (2005). Chronic Mild Stress Impact : are females more vulnerable? *Neuroscience*, *135*, 703–714.

Dalton-Jez, O. (2006). *The development of wheel-induced feeding suppression in male and female rats.* (Master's thesis). Retrieved from Scholars Common @ Laurier.

Davis, C. (1997). Eating disorders & hyperactivity: a psychobiological perspective. *Canadian Journal of Psychiatry, 42*, 168-75.

Doerries, L.E., Stanley, E.Z., & Aravich, P.F. (1991). Activity-based anorexia: Relationship, paradoxical or enigmatic? In W.F. Epling & W.D. Pierce (Eds). *Activity anorexia: Theory, research, and treatment.* (pp. 69-77). Mahwah, NJ: Erlbaum.

Eayrs, J.T. (1954). Spontaneous activity in the rat. *British Journal of Animal Behaviour, 2,* 25–30.

Eckel, L. A. (2011). The ovarian hormone estradiol plays a crucial role in the control of food intake in females. *Physiology & Behavior*, *104*, 517–524.

Eckel, L.A, Houpt, T.A,, & Geary, N. (2000). Spontaneous meal patterns in female rats with and without access to running wheels. *Physiology & Behavior*, *70*, 397–405.

Eckel, L.A. & Moore, S.R. (2004). Diet-induced hyperphagia in the rat is influenced by sex and exercise. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology*, *287,* 1080–1085.

Eikelboom, R., & Mills, R. (1988). A microanalysis of wheel running in male and female rats. *Physiology & Behavior*, *43*, 625–30.

Epling, W.F. & Pierce, W.D. (1988), Activity-based anorexia: A biobehavioral perspective. *International Journal of Eating Disorders, 7,* 475–485.

Epling, W.F., Pierce, W.D. & Stefan, L. (1983). A theory of activity-based anorexia. *International Journal of Eating Disorders, 3*, 27–46.

Fletcher, G., Balady, G., Blair, S.N., Blumenthal, J., Caspersen, C., Chaitman, B., Epstein, S., et al. (1996). Statement on Exercise: Benefits and Recommendations for Physical Activity Programs for All Americans. *Circulation, 94,* 857-862.

Frude, N. (1998). Eating Disorders. In *Understanding Abnormal Psychology: Basic Psychology* (pp.76-93). Oxford, England: Blackwell Publishing.

Gamer, D.M. & Garfinkel, P.E. (1997). *Handbook of treatment for eating disorders*. New York, NY: The Guilford Press.

Gross, C.G. **(**1968). General Activity. In: *Analysis of Behavioral Change* (Ed. by L. Weiskrantz), 91–106. New York: Harper & Row.

Hampstead, B.M., LaBounty, L.P. & Hurd, C. (2003). Multiple exposure to activity anorexia in rats: effects on eating, weight loss, and wheel running. *Behavioural Processes, 61*, 159-166.

Hancock, S., & Grant, V. (2009). Early maternal separation increases symptoms of activity-based anorexia in male and female rats. *Journal of Experimental Psychology: Animal Behavior Processes*, *35*(3), 394-406.

Hughes, R.L. (1964) Effect of changing cages, introduction of the male, and other procedures on the oestrous cycle of the rat. *CSIRO Wildlife Research, 9,* 115.

Ims, R.A. (1990). The ecology and evolution of reproductive synchrony. *Trends in Ecology & Evolution, 5,* 135-140.

Kagan, J. & Berkun, M. (1954). The reward value of running activity. *Journal of Comparative and Physiological Psychology, 47*(2), 108.

King, N.A., Burley, V.J., & Blundell, J.E. (1994). Exercise-induced suppression of appetite: effects on food intake and implications for energy balance. *European Journal of Clinical Nutrition, 48*(10), 715-724.

Koh, M.T., Lett, B.T., & Grant, V.L. (2000). Activity in the circular alley does not produce the activity anorexia syndrome in rats. *Appetite, 34,* 153–159.

Lattanzio, S.B., & Eikelboom, R. (2003). Wheel access duration in rats: I. Effects on feeding and running. *Behavioral Neuroscience*, *117*, 496–504.

Lau, D., Douketis, J.D., Morrison, K.M., Hramiak, I.M., & Sharma, A.M. (2007). 2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children [summary]. *Canadian Medical Association Journal*, *176*, S1–13.

Lett, B.T., Grant, V.L., Byrne, M.J., & Koh, M.T. (2000). Pairings of a distinctive chamber with the aftereffect of wheel running produce conditioned place preference. *Appetite*, *34*, 87–94.

Lopak, V., & Eikelboom, R. (2000). Pair housing induced feeding suppression: individual housing not novelty. *Physiology & Behavior*, *71*, 329–33.

Looy, H., & Eikelboom, R. (1989). Wheel running, food intake, and body weight in male rats. *Physiology & Behavior*, *45*, 403–5.

Martí, O., Martí, J. & Armario, A. (1994) Effects of chronic stress on food intake in rats: Influence of stressor intensity and duration of daily exposure. *Physiology & Behavior*, *55*, 747-753.

McClintock, M.K. (1978). Estrous synchrony and its mediation by airborne chemical communication (Rattus norvegicus). *Hormones and behavior*, *10*, 264–75.

McClintock, M.K. (1971). Menstrual Synchrony and Suppression. *Nature*, *229*, 244–245.

McClintock, M.K., Anisko, J.J. & Adler, N.T. (1982). Group Mating Among Norway Rats. *Animal Behaviour*, *30*, 398–409.

Mondon, C.E., Dolkas, C.B., Sims, C., Reaven, G.M., Mondon, C.E., & Reaven, M. (1985). Spontaneous running activity in male rats : effect of age. *Journal of Applied Physiology*, *58*, 1553–1557.

Mueller, D.T., Loft, A., & Eikelboom, R. (1997). Alternate-day wheel access: effects on feeding, body weight, and running. *Physiology & Behavior*, *62*, 905–908.

Nogal, P. & Lewiński, A. (2008). Anorexia Nervosa. *Endokrynologia Polska, 59*(2), 148-155.

O'Connor, R., & Eikelboom, R. (2000). The effects of changes in housing on feeding and wheel running. *Physiology & Behavior*, *68*, 361–71.

Patton G.C., Coffey, C., Sawyer S.M. (2003) The outcome of adolescent eating disorders: findings from the Victorian Adolescent Health Cohort Study. *European Child Adolescence Psychiatry, 12*(1), I25– I29.

Paré, W.P., Vincent, G.P., Isom, K.E., & Reevesm J.M. (1978). Sex differences and the incidence of activity stress ulcers in the rat. *Psychological Reports, 43,* 591-594.

Premack, D., & Premack, A.J. (1963). Increased eating in rats deprived of running. *Journal of the experimental analysis of behavior*, *6*, 209–12.

Richter, C.P. (1922). A behavioristic study of the activity of the rat. *Journal of Comparative Psychology Monographs, 1,* 1-55.

Robinson, T.E. (2004). Neuroscience. Addicted rats. *Science, 305*, 951–3.

Routtenberg, A., (1968). Self-starvation of rats living in activity wheels: adaptation effects. *Journal of Comparative Physiology Psychology, 66*, 234–238.

Ruis, M.A., te Brake, J.H., Buwalda, B., De Boer, S.F., Meerlo, P., Korte, S.M., & Koolhaas, J.M. (1999). Housing familiar male wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat. *Psychoneuroendocrinology*, *24*(3), 285–300.

Selye, H. (1976). Forty years of stress research: principal remaining problems and misconceptions. *Canadian Medical Association journal*, *115*(1), 53–56.

Sasse, S.K., Greenwood, B.N., Masini, C.V., Tara, J., Fleshner, M., Day, H.E., & Campeau, S. (2008). Chronic voluntary wheel running facilitates corticosterone response habituation to repeated audiogenic stress exposure in male rats. *Stress*, *11*(6), 425–437.

Schinckel, P.G. (1954). The effect of the ram on the incidence and occurrence of oestrus in ewes. *Australian Veterinary Journal*, *30*, 189–195.

Schwarzberg H. & Roth N. (1989). Increased locomotor activity of rats by self- stimulation in a running wheel. *Physiology & Behavior, 46*, 767-769.

Shelton, M. (1960). Influence of the Presence of a Male Goat on the Initiation of Estrous Cycling and Ovulation of Angora Does. *Journal of Animal Science*, *19*, 368–375.

Sherwin, C. (1998). Voluntary wheel running: a review and novel interpretation. *Animal Behaviour*, *56*, 11–27.

Siegfried, Z., Berry, E.M., Hao, S., & Avraham, Y. (2003). Animal models in the investigation of anorexia. *Physiology & Behavior*, *79*, 39–45.

Sullivan, P.F. (1995). Mortality in anorexia nervosa. *American Journal of Psychiatry, 152*, 1073-1074.

Stranahan, A.M., Lee, K., & Mattson, M.P. (2010). Central mechanisms of HPA axis regulation by voluntary exercise. *Neuromolecular Medicine*, *10*, 118–127.

Stewart, C.C. (1898). Variations in daily activity produced by alcohol and by changes in barometric pressure and diet with a description of recording methods. *American Journal of Physiology*, *1*, 40–56.

Strober, M. & Freeman, R. (1998). The long-term course of severe anorexia nervosa in adolescents: Survival analysis of recovery, relapse, and outcome predictors over 10–15 years in a prospective study. *International Journal of Eating Disorders, 22*, 339–360.

Tepper, J.S. & Weiss, B. (1986). Determinants of behavioral response with ozone exposure. *Journal of Applied Physiology*, *60*, 868–875.

Thompson, D.A., Wolfe, L.A. & Eikelboom, R. (1988). Acute effects of exercise intensity on appetite in young men. *Medical & Science in Sports & Exercise, 20*, 222-227.

Tokuyama, K., & Okuda, H. (1982). Effects of wheel running on food intake and weight gain of male and female rats, *Physiology & Behavior,28*, 899–903.

Tozzi, F., Thornton, L.M., Klump, K.L., Fichter, M.M., Halmi, K.A., Kaplan, A.S., et al. (2005). Symptom fluctuation in eating disorders: correlates of diagnostic crossover. *American Journal of Psychiatry, 162*,732–740.

Vallès, A., Martí, O., García, A., Armario, A., & Marti, O. (2012). Single exposure to stressors causes long-lasting, stress-dependent reduction of food intake in rats. *American Journal of Physiology, 279,* 1138-1144.

Van der Lee, S. & Boot, L.M. (1956). Spontaneous pseudopregnancy in mice. II. *Acta Physiologica et Pharmacologica Neerlandica,* 5, 213.

Wang, G.H. (1923). The relation between "spontaneous" activity and oestrous cycle in the white rat. *Comparative Psychology Monographs*, *2*, 1-27.

Westwood, F.R. (2008). The female rat reproductive cycle: a practical histological guide to staging. *Toxicologic Pathology*, *36*, 375–84.

Whitten, W.K. (1956). Modification of the oestrous cycle of the mouse by external stimuli associated with the male. *Journal of Endocrinology, 13*, 399–404.

Whitten, W.K**.** (1959). Occurrence of anoestrus in mice caged in groups. *Journal of Endocrinology*, 18, 102-107.

Whitten, W.K., Bronson, F.H. & Greenstein, J.A. (1968). Estrus-inducing pheromone of male mice: transport by movement of air. *Science, 161*, 584-585.

Woodside, D.B., Garfinkel, P.E., Lin, E., Goering, P., Kaplan, A.S., Goldbloom, D.S. & Kennedy, S.H. (2001). Comparisons of men with full or partial eating disorders, men without eating disorders and women with eating disorders in the community. *American Journal of Psychiatry*, *158*, 4.

World Health Organization. (2008). The WHO agenda. Retrieved March 2013, from http://www.who.int/mediacentre/factsheets/fs311/en/.

Figure Captions

Figure 1. Methodology of Replications 1 to 3. Replications 1 and 2 were run separately and both sexes were housed separately. **Daily measures of food intake, body weight & wheel turns were taken. ** Additional daily wheel turn measures were taken.*

Figure 2. Mean (± SEM) food consumption of Replication 1 unisex housed female rats from baseline through 30 days of wheel access for PW and IW compared to PNW and INW groups.

Figure 3. Mean (± SEM) wheel turns of Replication 1 unisex housed female rats for 30 days of wheel access for PW and IW groups.

Figure 4. Mean (± SEM) body weight of Replication 1 unisex housed female rats from baseline through 30 days of wheel access for PW and IW compared to PNW and INW groups.

Figure 5. Mean (± SEM) food consumption of Replication 2 unisex housed male rats from baseline through 30 days of wheel access for PW and IW compared to PNW and INW groups.

Figure 6. Mean (± SEM) wheel turns of Replication 2 unisex housed male rats for 30 days of wheel access for PW and IW compared to PNW and INW groups. *Note: technical errors in data collection occurred at the second block of running; rats still ran.*

Figure 7. Mean (± SEM) body weight of Replication 2 unisex housed male rats from baseline through 30 days of wheel access for PW and IW compared to PNW and INW groups.

Figure 8. Mean (± SEM) food consumption of Replication 3 female rats housed with males from baseline through 30 days of wheel access for PW and IW compared to PNW and INW groups.

Figure 17. Mean (± SEM) wheel turns and food intake for Replication 3 IW female rats, representing estrous cycle synchrony and regularity; *additional day E0 measurements for # of cages realigned and average # of days realigned/age.*

Appendix

Figure 1

Design for Replications 1-3

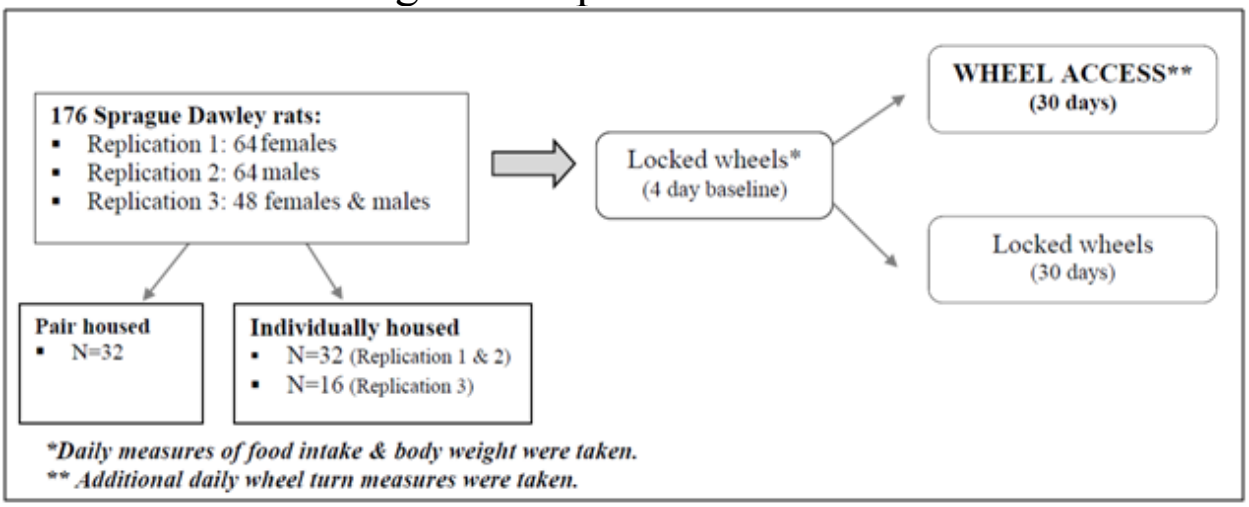


Figure 2

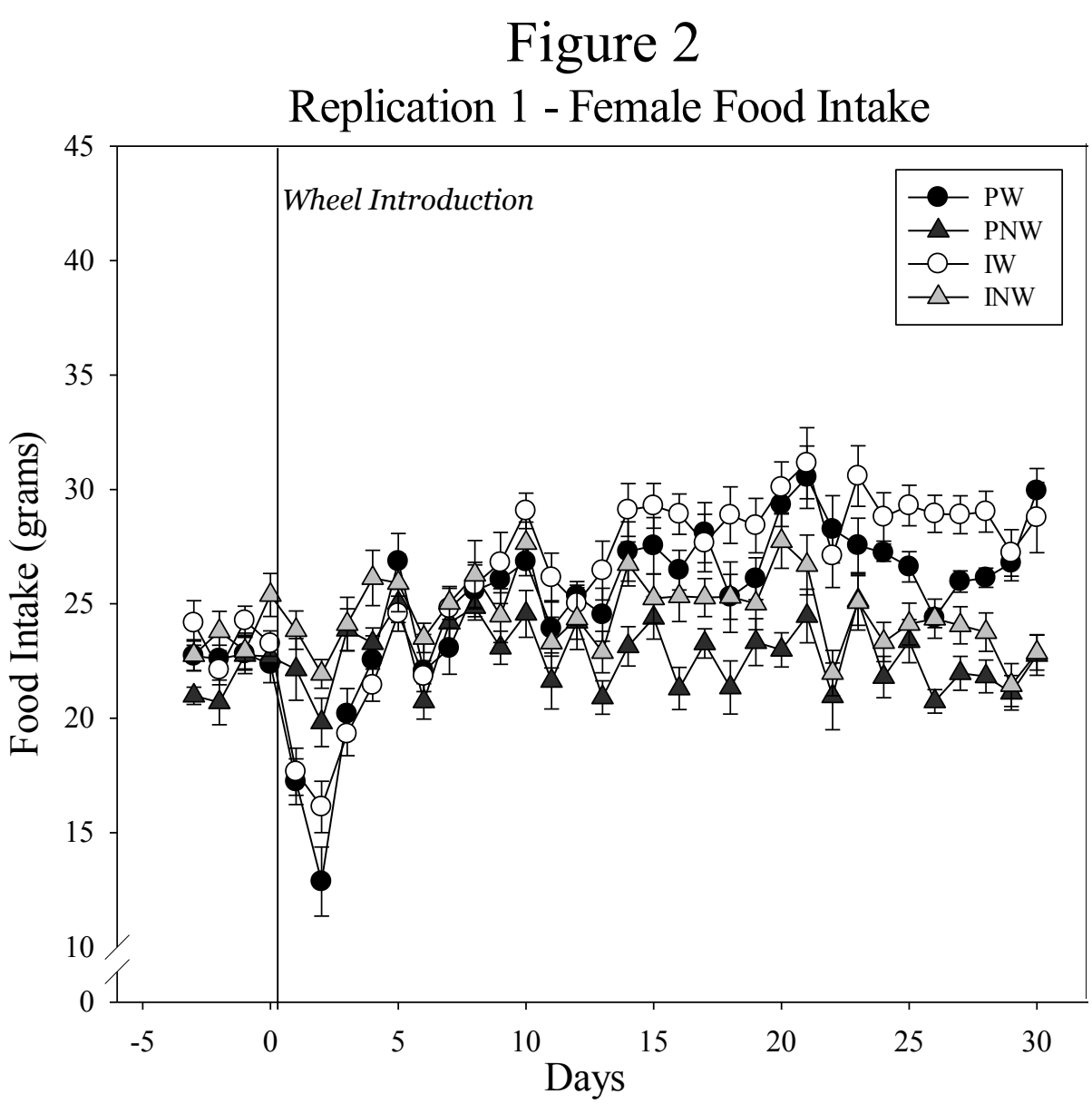

Replication 1 - Female Food Intake
Wheel Introduction
PW
PNW
IW
INW
Food Intake (grams)
Days
45
40
35
30
25
20
15
10
0
-5
0
5
10
15
20
25
30

Figure 3

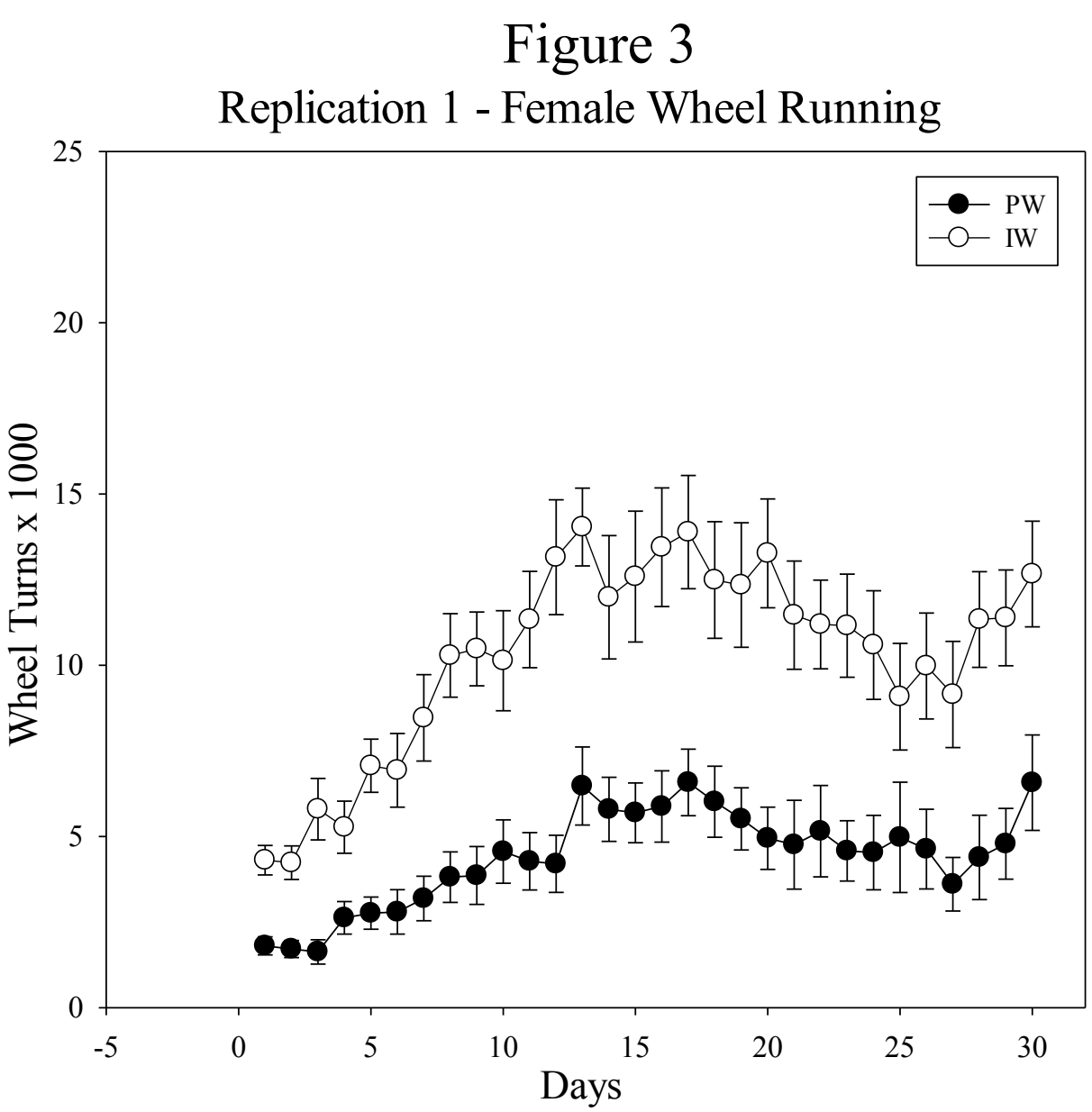

Replication 1 - Female Wheel Running
PW
IW
Wheel Turns x 1000
Days
0
5
10
15
20
25
-5
0
5
10
15
20
25
30

Figure 4

Replication 1 - Female Body Weight

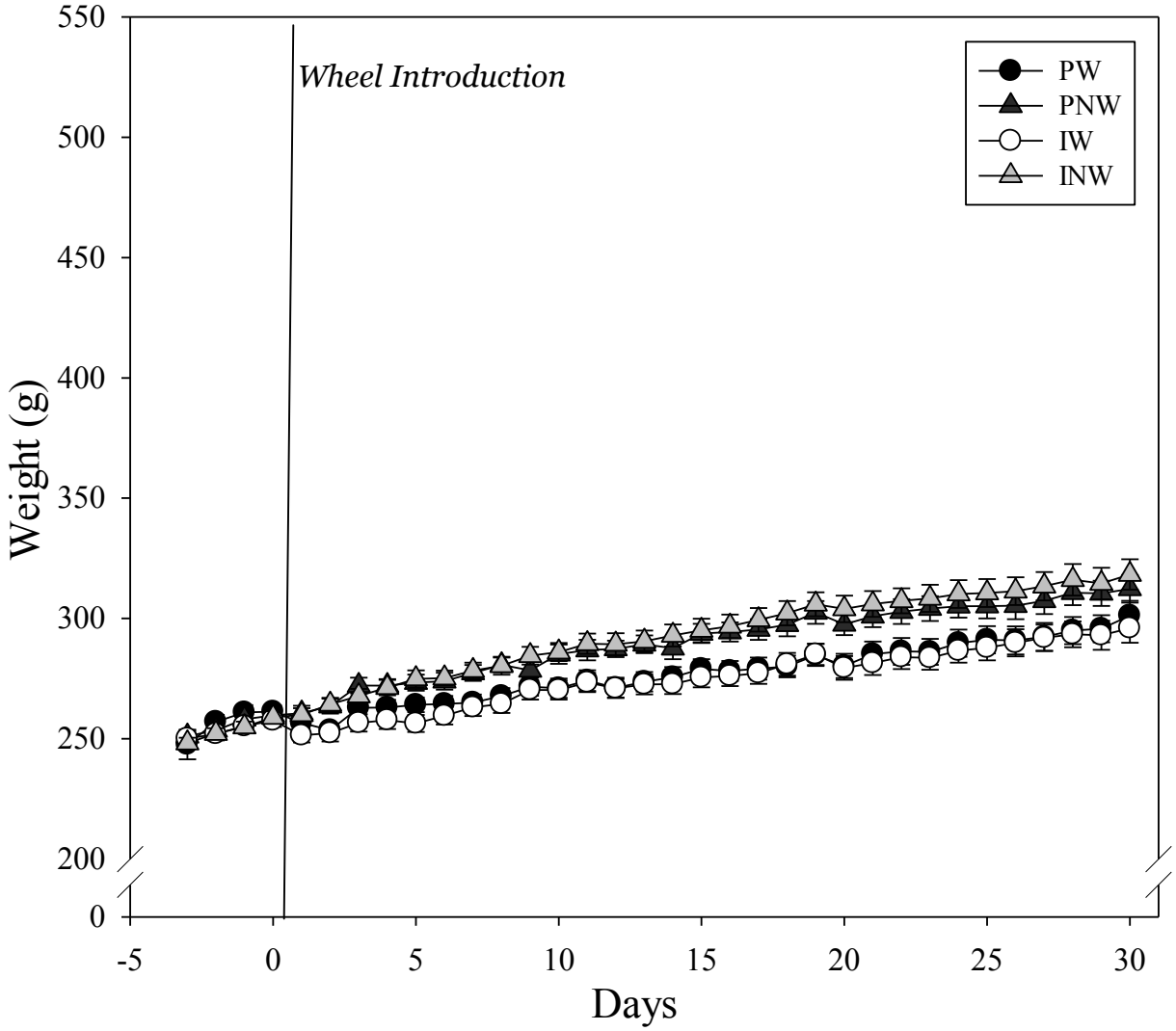


Figure 5

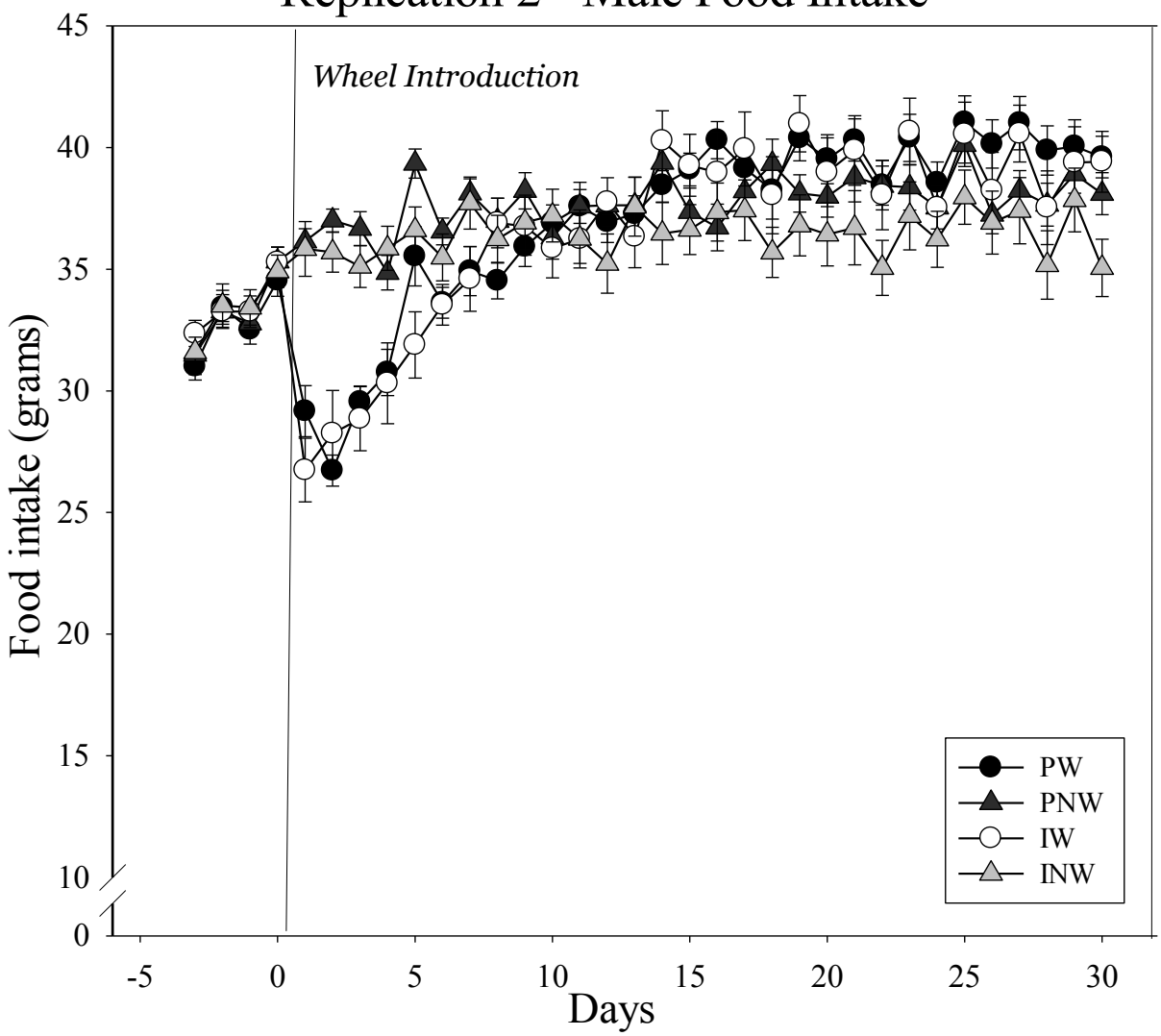

Replication 2 - Male Food Intake
Wheel Introduction
Food intake (grams)
Days
PW
PNW
IW
INW
45
40
35
30
25
20
15
10
0
-5
0
5
10
15
20
25
30

Figure 6

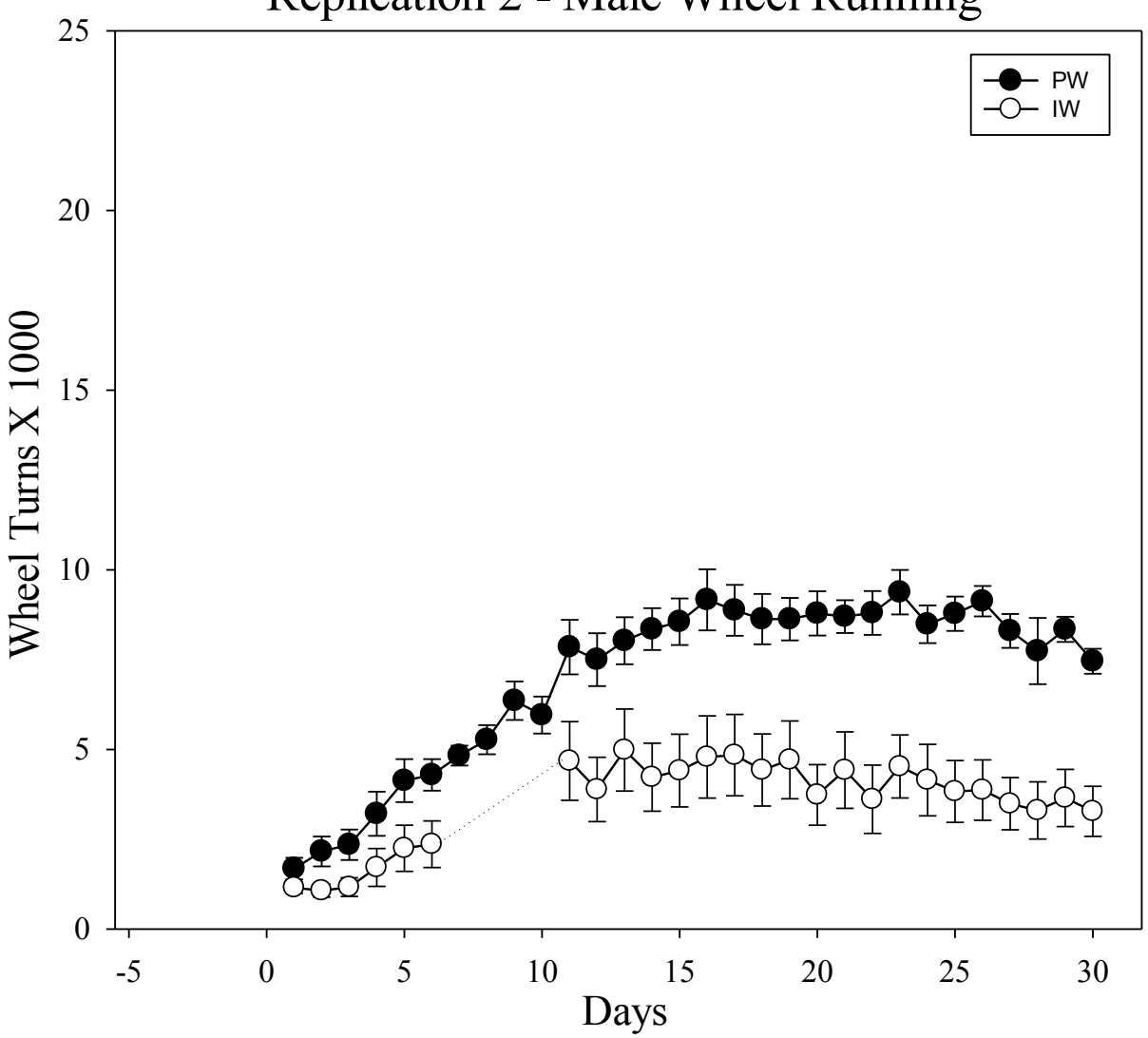

Replication 2 - Male Wheel Running
PW
IW
Wheel Turns X 1000
Days
25
20
15
10
5
0
-5
0
5
10
15
20
25
30

Figure 7

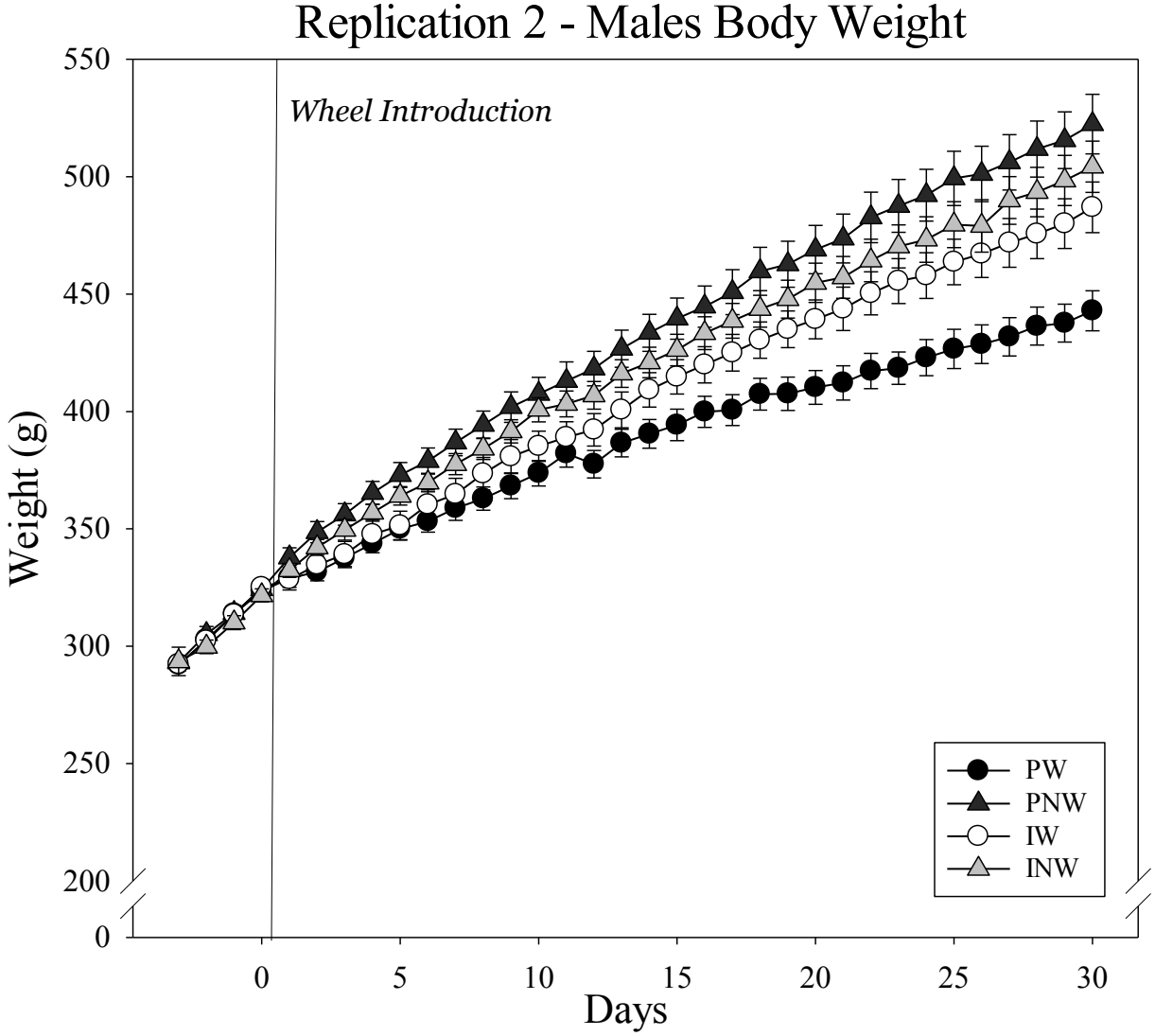

Replication 2 - Males Body Weight
Wheel Introduction
Weight (g)
550
500
450
400
350
300
250
200
0
0
5
10
15
20
25
30
Days
PW
PNW
IW
INW

Figure 8

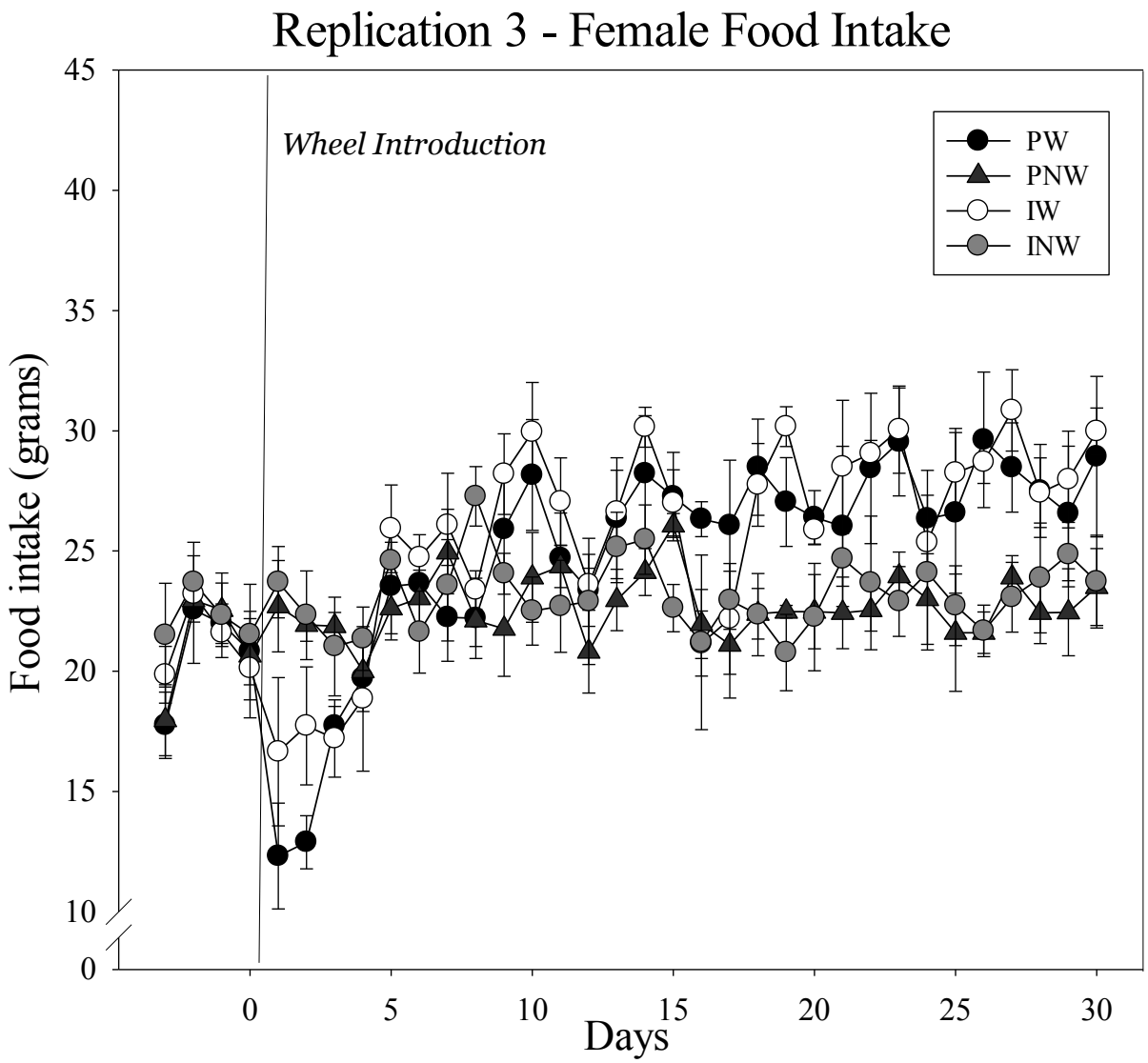

Replication 3 - Female Food Intake
Wheel Introduction
PW
PNW
IW
INW
Food intake (grams)
Days
45
40
35
30
25
20
15
10
0
0
5
10
15
20
25
30

Figure 9

Replication 3 - Female Wheel Running

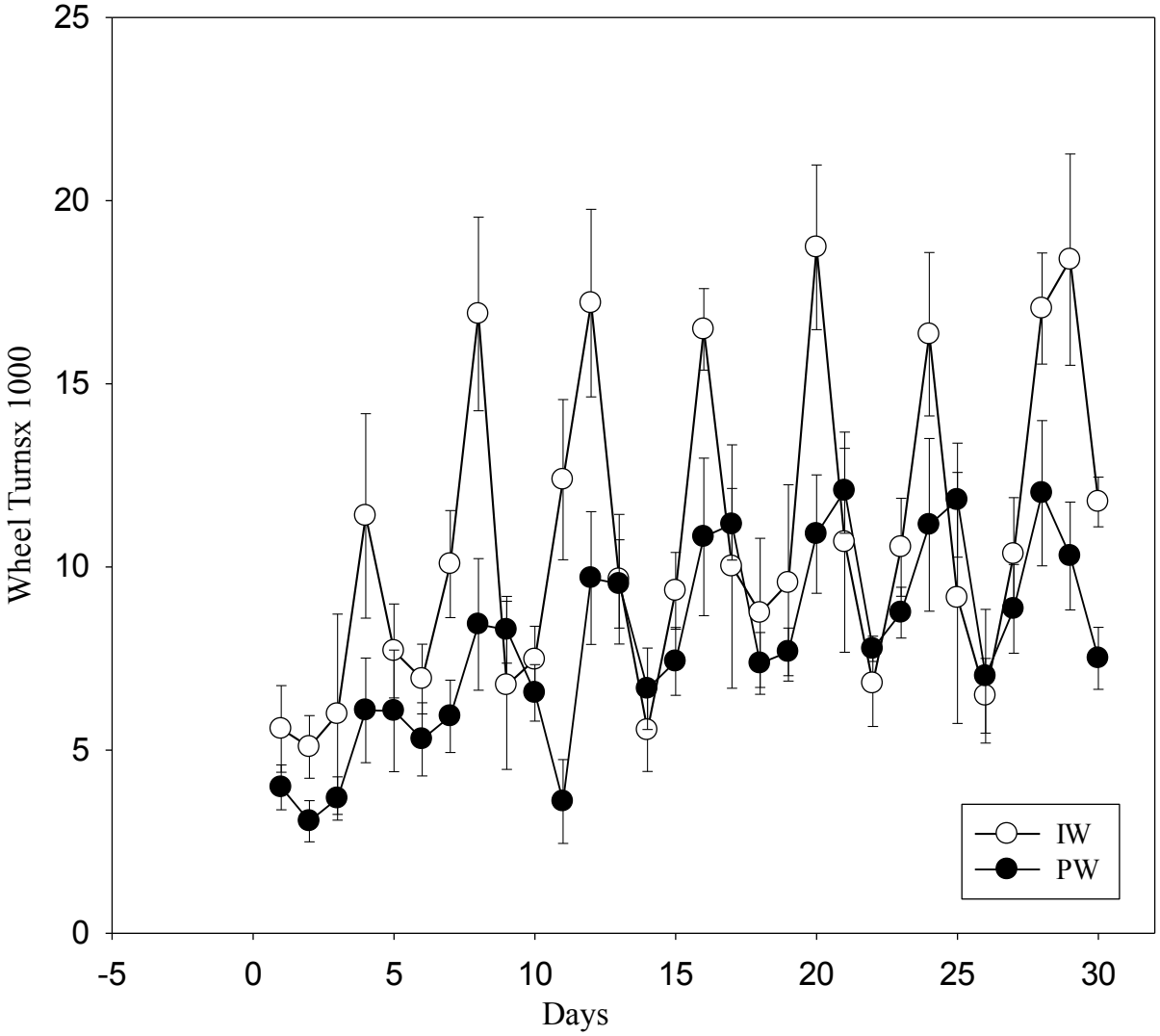


Figure 10

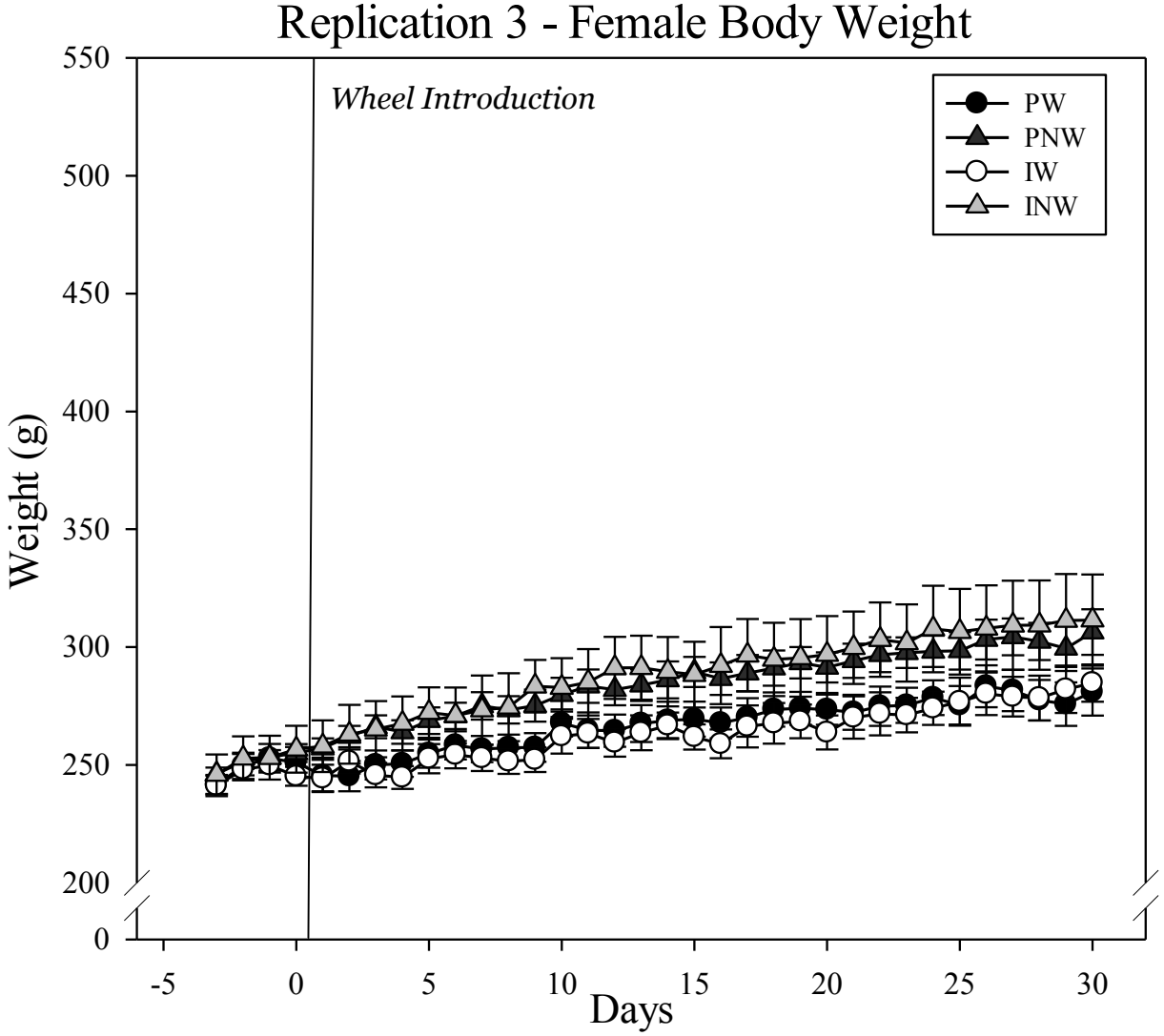

Replication 3 - Female Body Weight
Wheel Introduction
PW
PNW
IW
INW
Weight (g)
550
500
450
400
350
300
250
200
0
-5
0
5
10
15
20
25
30
Days

Figure 11

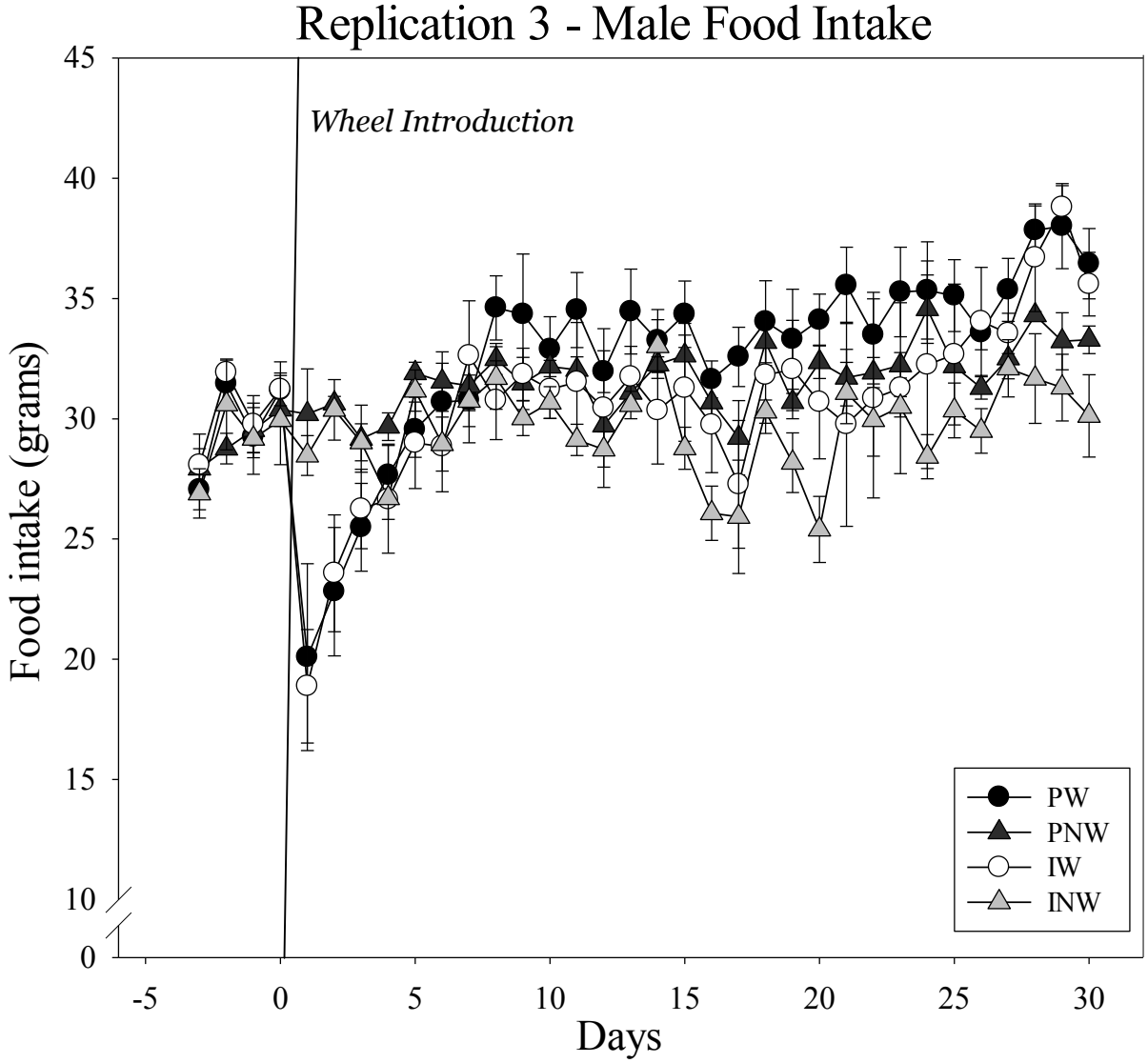

Replication 3 - Male Food Intake
Wheel Introduction
Food intake (grams)
Days
45
40
35
30
25
20
15
10
0
-5
0
5
10
15
20
25
30
PW
PNW
IW
INW

Figure 12

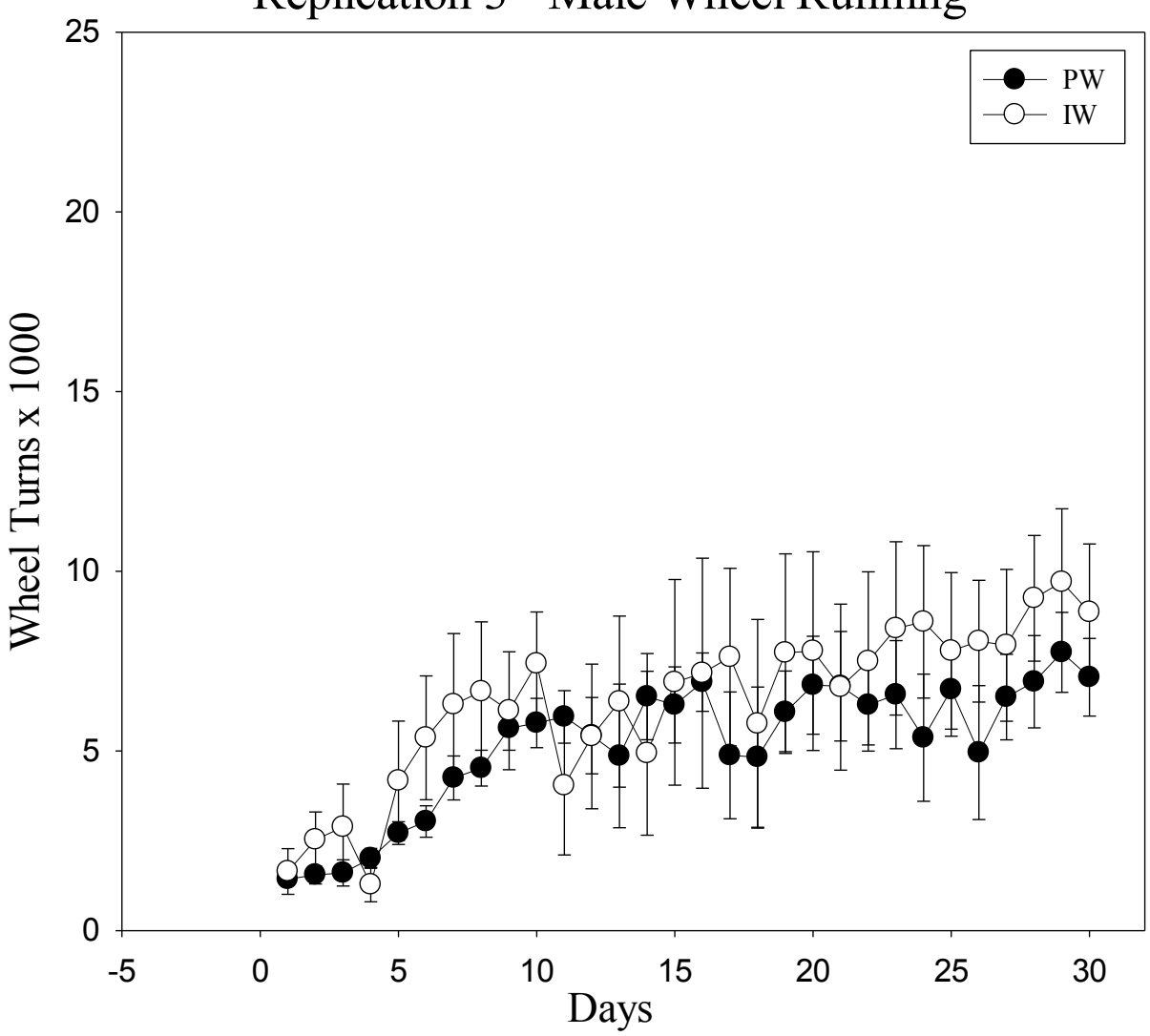

Replication 3 - Male Wheel Running
PW
IW
Wheel Turns x 1000
Days
0
5
10
15
20
25
-5
0
5
10
15
20
25
30

Figure 13

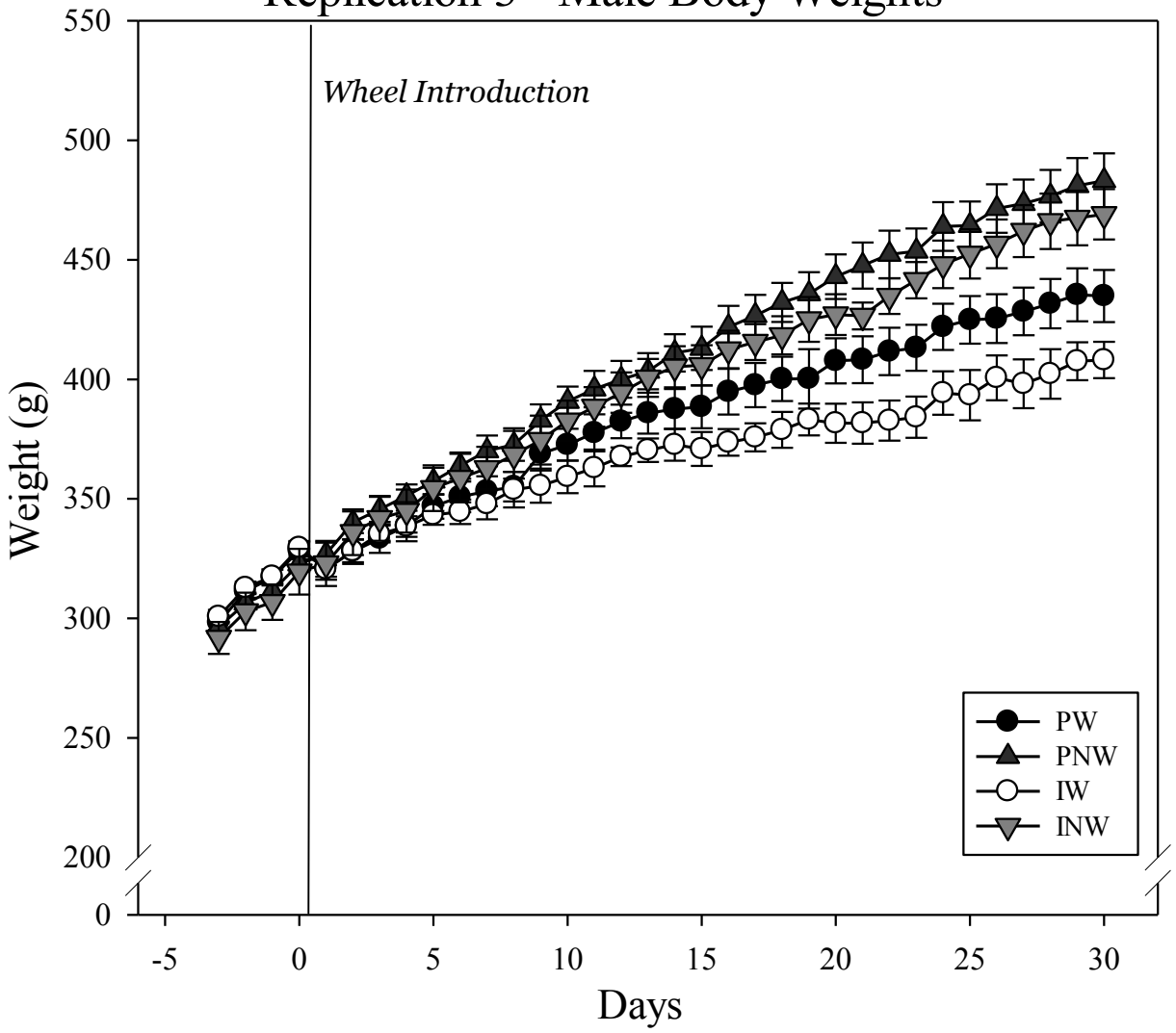
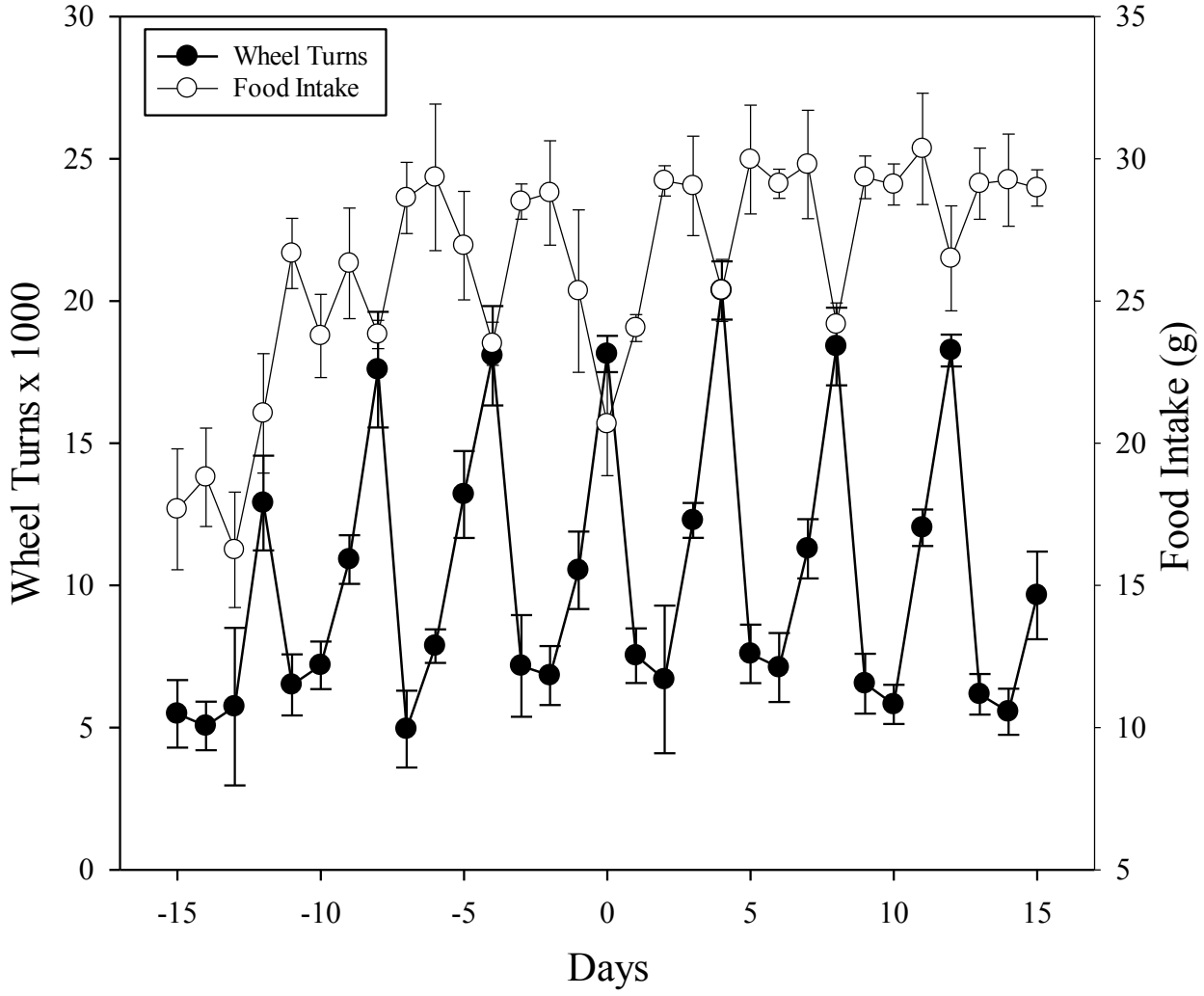

Replication 3 - Male Body Weights
Wheel Introduction
Weight (g)
550
500
450
400
350
300
250
200
0
-5
0
5
10
15
20
25
30
Days
PW
PNW
IW
INW

Figure 14

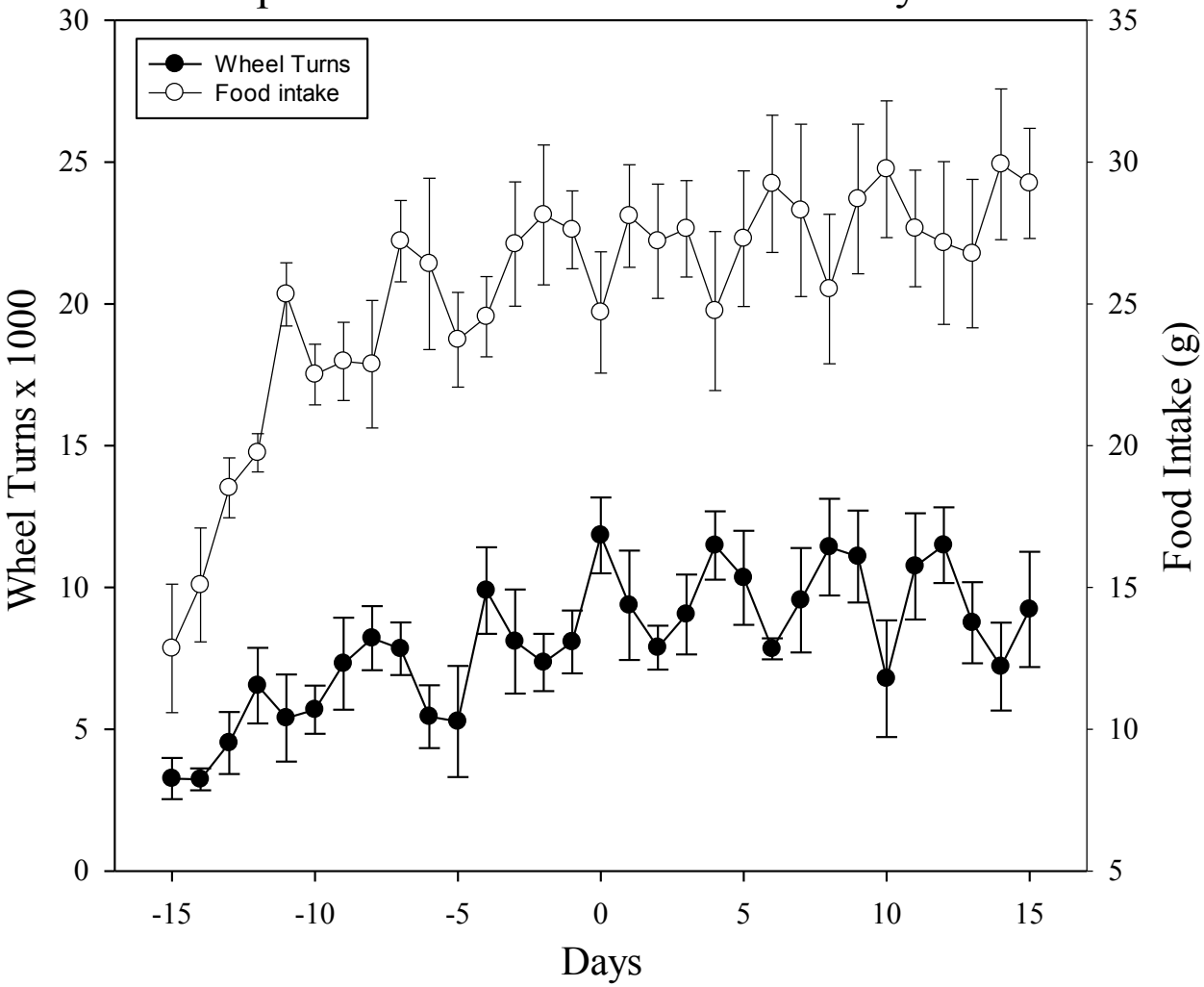
Replication 3 - Female IW Estrous Cycle



% Cages realigned	Average Days Realigned/Cage
25%	.25

Figure 15

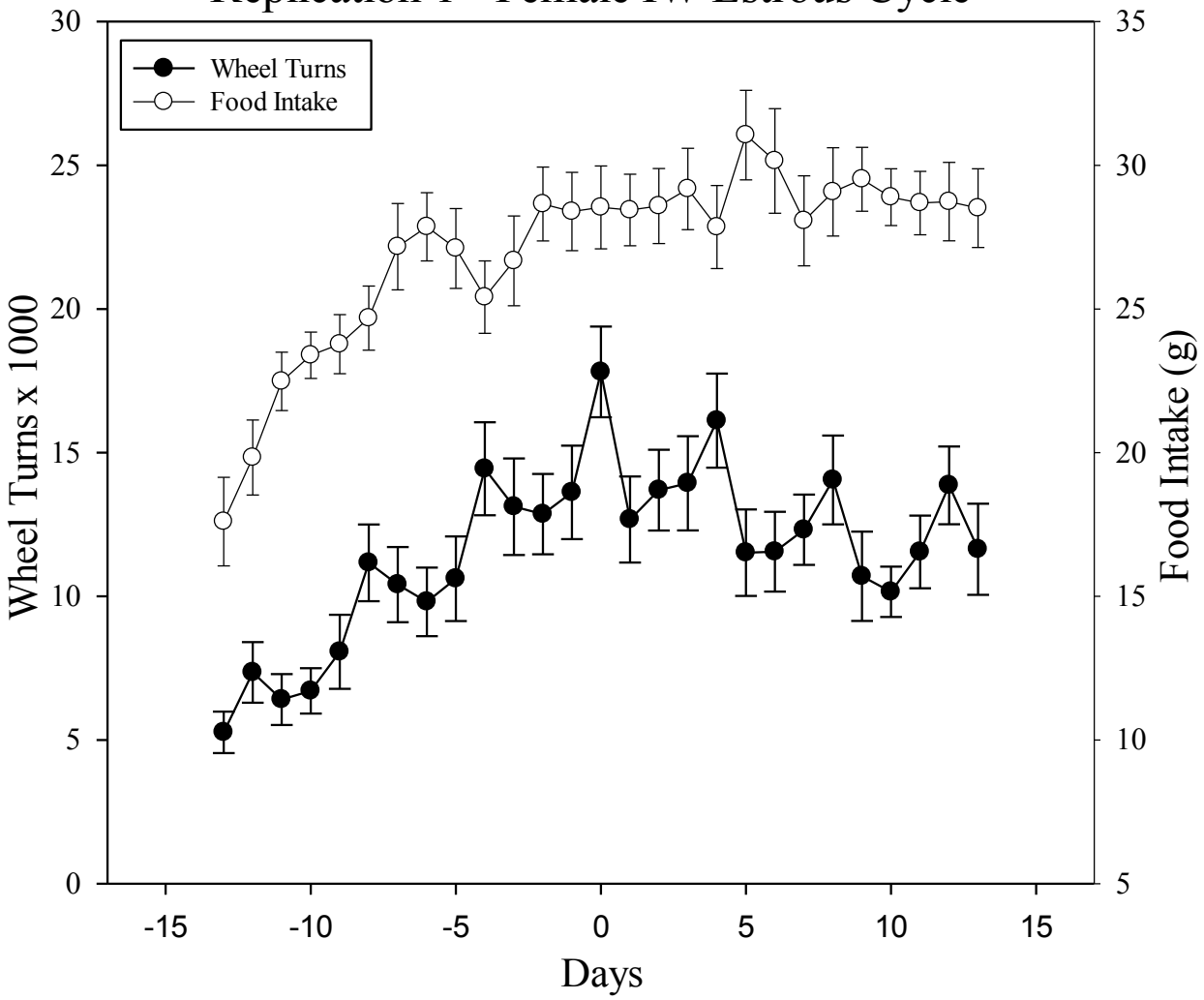
Replication 3 - Female PW Estrous Cycle



% Cages realigned	Average Days Realigned/Cage
50%	.50

Figure 16

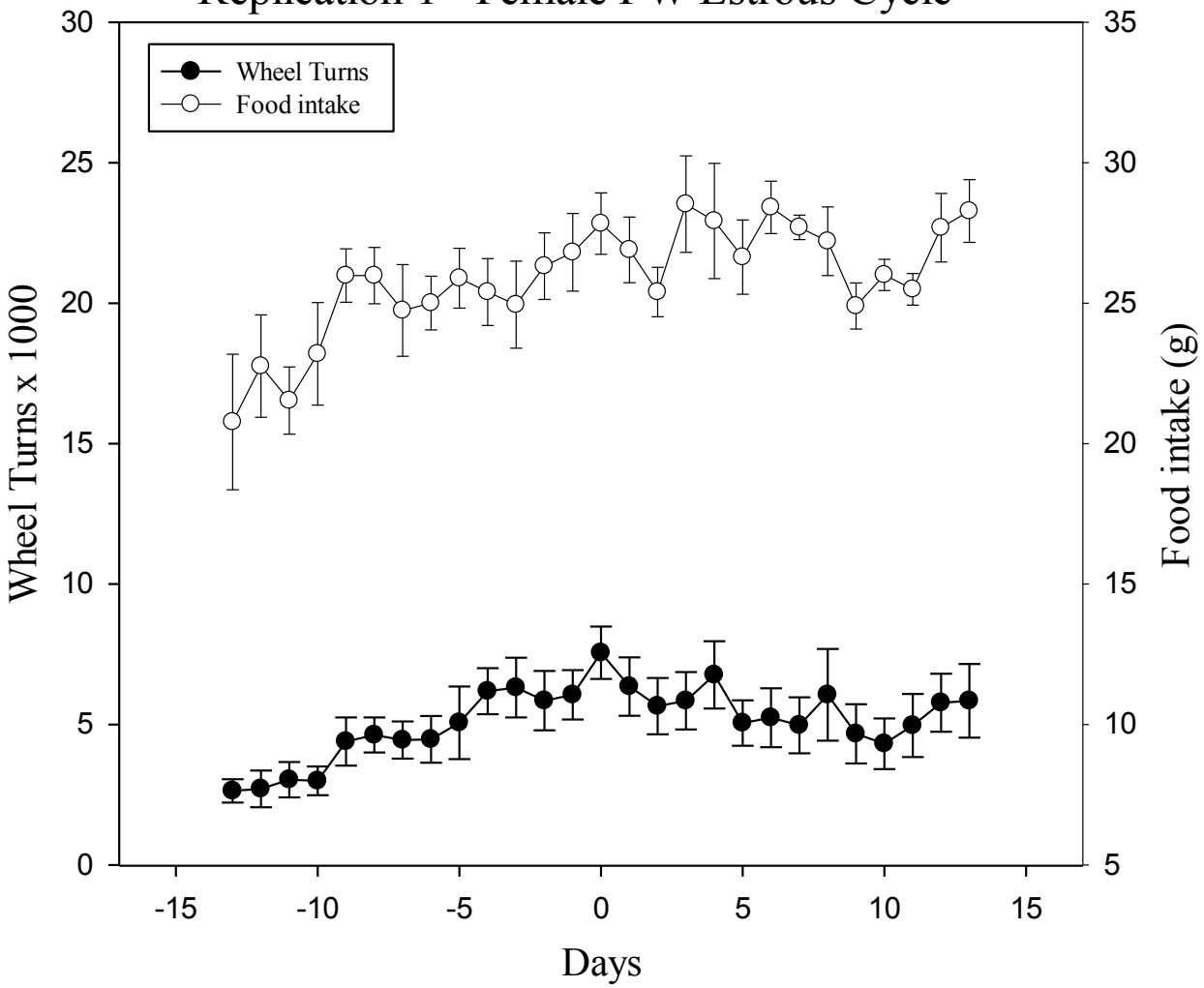
Replication 1 - Female IW Estrous Cycle



% Cages realigned	Average Days Realigned/Cage
69%	.85

Figure 17

Replication 1 - Female PW Estrous Cycle



% Cages realigned	Average Days Realigned/Cage
57%	1