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# Novelty and the running-induced feeding suppression

Elham Satvat
*Wilfrid Laurier University*

# Novelty and the Running-Induced Feeding Suppression

By

Elham Satvat

Bachelor of Art (Honours), Wilfrid Laurier University

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## Abstract

In adult rats, wheel introduction induces a temporary feeding suppression, which may be the result of either a conditioned taste avoidance induced by wheel running (Lett & Grant, 1996), or an anorectic effect produced by running (Mueller, Loft, & Eikelboom, 1997). The first experiment investigated the effect of alternate-day wheel access on consumption of novel 32% sucrose solution in 36 adult male rats. The first group of rats had no wheel access, the second continuous wheel access, and the third alternate-day wheel access. Rats without wheel access consumed large amounts of sucrose from the first day. Both groups with wheel access had similar, almost complete and long-lasting, suppressed sucrose consumption. The suppression occurred on both wheel and home cage days in the rats with alternate-day wheel exposure. This suggests that the unconditioned effect of running (whether "sickness" or some positive affect), when paired with a novel food, induces conditioned taste avoidance.

CS pre-exposure has been shown to reduce conditioned taste avoidance, possibly through latent inhibition (Lubow & Moore, 1959; Lubow, 1989). The second experiment investigated the effect of alternate-day wheel access on consumption of a familiar sucrose solution (rats were given 10 days sucrose pre-exposure) in a similar design. The rats with continuous wheel access showed only a mild and short-lived suppression of the sucrose solution. The rats with alternate-day wheel access showed a suppression of familiar sucrose on wheel days, possibly due to the unconditioned effect of running. However, the same rats did not show a suppression of familiar sucrose solution on home cage days perhaps due to the latent inhibition effect, which reduced the conditioned effect of wheel running. The results of these two experiments suggest that wheel running has an

unconditioned effect that directly influences food consumption. If the food is novel the suppression is more pronounced and lasts longer, because the unconditioned effect induced by running supports a conditioned taste avoidance.

Weanlings run as much as adult rats, but running does not induce a feeding suppression of a familiar food (Afonso, 2000). Thus, the third experiment investigated the effect of wheel access on consumption of a novel sucrose solution in 32 weanling, and 32 adult male rats. Each age was divided into four groups. Two groups had wheel access and two had sucrose access. Wheel running had a suppressive effect on consumption of familiar food and a more pronounced suppressive effect on consumption of a novel food in adult rats, but had no effect on consumption of both familiar and novel food in weanlings. The difference between weanling and adult rats on the effect of wheel running on feeding may be due to the rapid growth that weanlings are experiencing, or it may be due to the inability of younger rats to associate the novel food with the unconditioned effect of running. Taken together, the results suggest that food novelty makes the feeding suppression induced by wheel running much stronger and that this feeding suppression is not evident in young rats.

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# Table of Contents

# Lists of Figures

Experiment 1:



Experiment 2:

Experiment 3:

# Novelty and the Running-Induced Feeding Suppression

Rats will voluntarily and spontaneously run soon after being given access to the wheels (Stewart, 1898; Richter, 1927). A substantial body of evidence demonstrates that running in the wheel influences energy balance in adult male and female rats (Levitsky, 1970; Looy & Eikelboom, 1989; Premack & Premack, 1963). With running, energy expenditure increases and normally an elevation in food intake might be expected. Paradoxically, when a wheel is first made available to rats, a noticeable suppression in food intake is evident (Afonso, 2000; Afonso & Eikelboom, 2003; Bauman, 1992; Levitsky, 1970; Looy & Eikelboom, 1989; Mueller, Loft, & Eikelboom, 1997; Premack & Premack, 1963). Both males and females suppress their food consumption to the same degree after wheel introduction (Afonso, 2000). There have been two explanations for this running-induced feeding suppression. It may be the result of a conditioned taste avoidance generated by wheel running (Lett & Grant, 1996), or it may be the result of an anorectic effect produced by running (Mueller et al., 1997). It is also possible that both of these effects (anorectic and conditioned taste avoidance) act in concert to produce the intake suppression.

## *Wheel Running and Conditioned Taste Avoidance*

In a number of studies, the food suppression induced by wheel running has been suggested to be the result of a conditioned taste avoidance/aversion (the terms have been used interchangeably in the literature, but the more descriptive avoidance will be used in this thesis) (Hayashi, Nakajima, Urushihara, & Imada, 2002; Lett & Grant, 1996; Lett, Grant, & Gaborko, 1998; Lett, Grant, Koh, & Smith, 2001; Nakajima, Hayashi, & Kato, 2000). Taste avoidance is a common type of Pavlovian conditioning procedure.

Typically, water deprived animals are presented with a distinctive flavoured solution, which serves as a conditioned stimulus (CS). After a period of drinking, animals are injected with an illness inducing agent, such as LiCl, which serves as an unconditioned stimulus (US). After a few conditioning trials, pairing the CS and US, the animals are presented with a choice between the flavoured solution and tap water. Animals will avoid the flavoured solution even after only one conditioning trial. Animals come to associate a taste (CS) with the following aversive state (US), hence avoiding the taste next time they encounter it (Domjan & Wilson, 1972; Garcia & Koelling, 1966).

Lett and Grant (1996) used wheel running as an unconditioned stimulus to produce a conditioned taste avoidance. Food and water deprived rats were given 10 minutes access to a flavoured solution. Following this, the experimental group rats were given half an hour wheel access while the control rats stayed in the home cages. After two conditioning trials, the experimental group animals avoided the flavoured solution, while this avoidance was not evident in the control animals. Lett and Grant (1996) suggested that wheel running may produce "sickness" as a side effect. The animals associated the sickness with the novel taste and avoided consuming the flavoured solution. It was suggested that this taste avoidance effect was responsible for the running-induced feeding suppression. In a follow-up study, it was shown that a similar taste avoidance could be elicited in rats not deprived of food and water (Lett et al., 1998).

Heth, Inglis, Russell, and Pierce (2001) explored whether the conditioned taste avoidance was induced by running per se or by the novel wheel environment. In their study, for three groups of rats, flavoured solutions preceded one of three conditions: 60 minutes access to unlocked wheels, 60 minutes access to locked wheels, or remaining in

the home cages. Only the rats that had an opportunity to run developed a taste avoidance. The authors thus confirmed that running itself rather than the novel wheel environment induced conditioned taste avoidance.

Repeated exposure to the US, before conditioning trials, has been shown to disrupt CS-US association (Best & Domjan, 1979; Cole, Bakner, Vernon, & Riccio, 1993; Randich & LoLordo, 1979; Riley & Simpson, 2001). For instance, when an animal is injected with the emetic drug LiCl several times, before introduction of CS-injection pairings, the rat fails to develop an association between the CS (flavoured solution) and the illness (Best & Domjan, 1979; Riley & Simpson, 2001). The reason for this disruption of CS-US association by US pre-exposure is thought to be due to associative blocking. Contextual cues are associated with the US during US pre-exposure. Thus, once the CS is introduced, the same contextual cues block the association of the target flavoured solution with the US (Riley & Simpson, 2001).

Salvy, Pierce, Heth, and Russell (2002) investigated the effect of wheel running (US) pre-exposure on conditioned taste avoidance learning in three groups of rats. One group of rats was pre-exposed to free turning wheels and another group of rats to locked wheels for one hour a day for seven days. The third group of rats had no wheel exposure and remained in their home cage. Food and water access were then restricted and conditioning trials started, during which time all rats were given half an hour access to a flavoured solution, followed by one hour wheel access. A second flavoured solution was also offered the day after, but was not followed by wheel access. On the test day only the rats pre-exposed to the free turning wheels failed to show a taste avoidance. Since wheel pre-exposure attenuated the taste avoidance, it was argued first that, wheel running

induced a conditioned taste avoidance through Pavlovian conditioning, because running induced a taste avoidance in the two groups with no prior running experience. Second, for wheel running to induce conditioned taste avoidance, running needed to be novel. Based on the associative blocking explanation, perhaps environmental cues were associated with wheel running in the group of rats with prior wheel access. When the novel taste was introduced, the environmental cues blocked the formation of association between the taste and the wheel running. Therefore, the rats in the pre-exposure group failed to show a taste avoidance to the novel flavoured solution.

There is a positive correlation between the strength of the conditioned taste avoidance and the US intensity. When a higher dose of an illness inducing agent is paired with a flavoured solution, a stronger conditioned taste avoidance is observed than if a lower dose of the same agent is used (Andrews & Braveman, 1975; Dragoin, 1971). The strength of taste avoidance induced by different amounts of running has also been studied. Sixty minutes of wheel access has been shown to induce a stronger taste avoidance to a novel flavoured solution than does 15 minutes of wheel access (Hayashi et al., 2002).

Temporal contiguity is also an important factor in the establishment of a conditioned taste avoidance. There is a negative relationship between the CS-US interval and the strength of the conditioned taste avoidance. The longer the CS-US interval used, the weaker the conditioned taste avoidance developed. Conversely, the shorter the CS-US interval, the stronger the conditioned taste avoidance becomes (Andrews & Braveman, 1975; Nachman, 1970). Similarly, it has been demonstrated that immediate wheel access

after drinking is more efficient in producing taste avoidance than is delayed access (Hayashi et al., 2002).

In all of the above studies (Hayashi et al., 2002; Heth et al., 2001; Lett & Grant, 1996; Lett et al., 1998; Lett, Grant, Koh, & Smith, 2001; Nakajima et al., 2000; Salvy et al., 2002), the traditional procedure for conditioning has been employed, which involves limited access to both the CS (flavoured solution) and the US (wheel running). Based on this classical conditioning procedure, Lett and Grant (1996) argued that ad lib feeding suppression induced by chronic wheel access may also be the result of conditioned taste avoidance. They proposed that wheel running may have nausea-inducing properties that make the animals "sick". If food is available after running, the running-induced nausea may directly suppress the food consumption of the animals. The running-induced nausea may also indirectly support a conditioned aversion to the food by means of sickness-based conditioning. As pointed out, temporal contiguity (the CS-US interval) is an important factor in establishment of a conditioned taste avoidance. In the case of the ad lib wheel and food access, it is not clear exactly how temporal contiguity of running and food consumption occurs. Whether the animals consume their food immediately after running or delay their consumption for a period of time after running has yet to be investigated. If the animals consume their food immediately prior to wheel running, then formation of a taste avoidance as proposed by Lett and Grant (1996) is possible. However, if the animals start to run and then consume their food sometime after running, this relationship is less likely to support a taste avoidance. Another alternate explanation for this effect is that running induces an anorectic state that suppresses appetite resulting in intake suppression (Mueller et al., 1997).

*Wheel Running Induces an Anorectic Effect*

In the case of ad lib wheel and food access, feeding is suppressed by up to 40% when rats are first introduced to running wheels (Afonso & Eikelboom, 2003). However, the feeding suppression disappears after about 10 days, and the rats gradually increase their food intake to a level similar or more than the intake of control rats (Afonso, 2000; Afonso & Eikelboom, 2003; Looy & Eikelboom, 1989; Mueller et al. 1997). Nonetheless, a physiological consequence of the initial feeding suppression induced by wheel running is weight loss and a slower rate of weight gain. Even though the rats increase their food consumption by the second week following the wheel introduction, their body weight never returns to that of control rats, at least for as long as they have access to wheel (Afonso, 2000; Bauman, 1992; Levitsky, 1970; Looy & Eikelboom, 1989; Mueller et al. 1997).

In a recent study, Lattanzio and Eikelboom (2003) explored the effects of limited periods of wheel running on ad lib food consumption. The same degree of initial feeding suppression and weight loss was seen in rats with ad lib and in rats with the two hours a day of wheel access. This suggests that the running-induced feeding suppression and the resulting weight loss does not require ad lib wheel running, and that the amount of running necessary to induce an intake suppression need not be large. Since rats eat mostly at night the limited day time wheel access is also temporally disconnected from the feeding. This suggests difficulties for a taste avoidance explanation of the feeding suppression.

It is not clear why, in an ad lib feeding situation, running induces a feeding suppression. Studies indicate that, while important, novelty of wheel running is not the

sole factor responsible for the running-induced feeding suppression, and that running may induce an anorectic effect leading to the disruption of appetite and a suppression of food intake, even in rats with prior wheel experience. For example, if after extensive wheel experience rats are deprived of running for 10 days and are then re-introduced to the running wheels, the running-induced feeding suppression is again evident (Looy & Eikelboom, 1989, Mueller, Herman, & Eikelboom, 1999).

Clearly, a complex interaction exists between wheel running and feeding. Mueller et al. (1997) explored the effects on feeding on alternate-day wheel access. One group of rats was kept in their home cages, another group of rats was given continuous access to running wheels, and the final group of rats was given alternate-day wheel access. For the home cage rats, food consumption remained constant over the 32 days of the experiment, while the group of rats with continuous wheel access showed an initial feeding suppression that recovered after about 10 days. The most fascinating pattern of food consumption was observed in the rats with alternate-day wheel exposure. For rats in this group, there was a significant difference between the food consumption on wheel days and on non-wheel, home cage days. In other words, rats suppressed their food intake only on the days on which they had access to the wheels, while on the intervening days with no wheel access, they consumed as much as the control rats. The rats with alternate-day wheel exposure had a total of 16 days of wheel running over 32 days of the study, and their feeding was suppressed each time the rats were given wheel access. Note, however, that the consumption of the rats with continuous wheel access recovered after 10 days of wheel running.

The results of the Mueller et al. (1997) alternate-day wheel exposure study suggest that running may induce an anorectic effect that suppresses appetite, causing the rats to consume less food when given wheel access. Whereas the rats with alternate-day wheel access had the opportunity to restore their energy balance on the intervening non-wheel days by consuming more food, the rats with continuous wheel access did not have a chance to restore their energy balance. Perhaps the combined effects of weight loss and elevated energy expenditure due to running increased hunger over days for the rats with continuous wheel access, so that the animals recovered their food intake after about 10-days. The group with alternate-day wheel access, however, had intervening non-wheel days to restore their energy balance, thus the animals' suppression of food on wheel-days was maintained throughout the study. Additionally this study suggests that the effect of the running-induced feeding suppression is evident for only about 24 hours, since rats do not show the feeding suppression on the second day when the wheels are not available to them.

Taken together, it is possible that running induces an anorectic effect that directly influences and suppresses appetite, causing animals to consume less food when they are introduced to running wheels.

*Effect of Wheel Running on Novel Food Consumption*

Since food novelty is important in taste avoidance learning, it is important to consider the effects of wheel access on novel food consumption. A number of studies have looked at the effect of wheel running on consumption of an ad lib novel food rather than a novel flavoured solution (Jennings & McCutcheon, 1974; Nikoletseas, 1981;

Satvat & Eikelboom, 2003). As the focus of these studies was food consumption, a 32% sucrose solution (i.e. calorie rich food) was used instead of a non-nutritive saccharin or other flavoured solution.

In Jennings and McCutcheon's 1974, study, one group of rats were given ad lib access to the novel 32% sucrose solution at the introduction of ad lib wheel access. The control group of rats without wheel access were also given ad lib sucrose access. The group of rats with wheel access suppressed their sucrose intake compared to the rats in the control group. On day 19, wheel access was switched between the two groups by moving the rats in the wheels into wheel-less home cages and placing the rats in the home cages into the cages equipped with running wheels. The control group rats, who had 18 days of sucrose pre-exposure but no prior wheel access, suppressed their sucrose consumption at wheel introduction. The rats in the experimental group increased their sucrose consumption, once wheels were removed. In a second experiment, Jennings and McCutcheon (1974) explored whether the running-induced sucrose suppression was only evident with novel wheels. Thus, consumption of the novel sucrose solution of the rats with and without prior wheel running experience was compared. The experimental animals had 19 days of ad lib wheel access while the control animals were kept in their home cages. On day 20, rats in the control group were also introduced to running wheels, and both groups were introduced to an ad lib 32% sucrose solution for the next 3 days. There was a significant difference in consumption of the novel sucrose between the rats with wheel pre-exposure and that of the rats with no prior wheel running experience. The rats with wheel pre-exposure consumed more amount of sucrose compared to the other

group of rats. It was concluded that the combination of both a novel food and a novel wheel induced neophobia and hence the inhibition of sucrose consumption.

Nikoletseas (1981) looked at the 32% sucrose consumption of four female rats that were 4-month old in a very complex design, reporting only 5 days average consumption. After a ten-day baseline period of ad lib sucrose access, the rats were transferred to activity wheels for one hour a day for 20 days. The animals had access to ad lib food, 32% sucrose solution and water in their home cages. Compared to baseline, sucrose consumption was suppressed during the 20 days that rats had opportunity to run for one hour a day (there was no control group in this experiment without wheel access). This period was followed by a 10-day sedentary phase, during which the animals were placed in the running wheel cages as before for one hour a day, but access to the wheels was barred. Sucrose consumption increased and returned to baseline levels during this phase. After this 10 day sedentary period, the animals were re-housed in the wheels and had the opportunity to run for 24 hours a day for 5 days. Sucrose solution, rat chow and water were still available ad lib. Sucrose consumption was again noticeably suppressed. Nikoletseas (1981) argued that, exercise, whether for one hour or 24 hours a day, results in a suppression of sucrose and calorie intake. Because the suppression of sucrose consumption in this study occurred in a familiar environment, Nikoletseas (1981) disagreed with the neophobia explanation for the wheel-induced sucrose suppression, suggested by Jennings and McCutcheon (1974). Note that a 10 day sedentary period in Nikoletseas' experiment intervened between the periods of one and 24 hour wheel access. In subsequent work it has been shown that access to running wheels, whether for a few hours a day or 24 hours a day, induced an initial feeding suppression of familiar food,

which lasted for about a week (Lattanzio & Eikelboom, 2003). In addition, when wheels were not available for 10 days and were then re-introduced, the feeding suppression was again evident (Mueller et al, 1999). Thus, these aspects of Nikoletseas' results have been confirmed and it is clear that neophobia is not responsible for the intake suppression induced by wheel running.

Even though both of these studies (i.e. Jennings & McCutcheon, 1974; Nikoletseas, 1981) show interesting interactions between the wheel access and novel food consumption, they have limitations. Nikoletseas (1981) used only 4 rats in his study and did not have any controls. Jennings and McCutcheon's (1974) study also lacked appropriate control groups with only familiar food access, given wheel or no-wheel experience. Moreover, in this study, only intake of the novel sucrose was reported, however the rats also had access to rat chow; it is not clear what the effect of running was on consumption of familiar food. In other words, it is not apparent from this study whether wheel access suppressed consumption of the familiar food or suppressed only consumption of the novel food.

In a more recent study the relationship between a novel food and wheel running was further explored (Satvat & Eikelboom, 2003). Because the interest was in novel food consumption, consistent with previous studies, (Jennings & McCutcheon, 1974; Nikoletseas, 1981) a 32% sucrose solution was used. Four groups of rats were given ad lib access to either the sucrose solution, the running wheel or both. All the rats were also given ad lib access to tap water and familiar rat chow throughout the study. After a 7-day habituation period, rats were placed in their wheels or home cages, and half of the animals were introduced to a 32% sucrose solution (the four groups were: Wheel-

Sucrose, Wheel-noSucrose, noWheel-Sucrose, and noWheel-noSucrose). This phase of the experiment lasted for 25 days, allowing the wheel running of the rats to reach a plateau. The two groups of rats with wheel access (Wheel-Sucrose and Wheel-noSucrose) showed a similar temporary (about 6 days) suppression of familiar rat chow intake. The two groups with sucrose access (Wheel-Sucrose and noWheel-Sucrose) showed a significant difference in sucrose intake. Whereas the rats with no wheel access consumed a large amount of the sucrose solution from the first day of sucrose availability, the rats with wheel access showed an almost complete suppression of sucrose consumption. It took about 2 weeks before sucrose consumption in this group of rats started to increase. By day 25 the rats in both groups were consuming similar amounts of sucrose solution. Therefore, wheel running not only induced a noticeable suppression of familiar rat chow, but it also induced a much more pronounced and longer lasting suppression of the novel sucrose solution.

The second phase of the study involved introducing the sucrose to the other two groups of rats, which were not given sucrose access in the first phase (Wheel-noSucrose and noWheel-noSucrose). No difference was found in the sucrose consumption of these two groups. In fact, all four groups of rats were consuming similar amounts of sucrose in the second phase of the study. The findings, were consistent with previous studies (Jennings & McCutcheon, 1974; Nikoletseas, 1981), and suggest that wheel introduction suppresses consumption of both familiar rat chow and novel sucrose solution but its suppressive effect is more pronounced and longer lasting with the novel sucrose. Further, novelty of the wheel running is critical for the suppression of any food intake, because

when rats are experienced with running (with no gap), their novel sucrose consumption resembles that of control rats.

Suppression of the novel food intake in the above studies can be explained in terms of a conditioned taste avoidance. Running perhaps induces a different physiological state (either negative or positive), that becomes associated with the novel food, and hence, the animals avoid consumption of this novel food.

## Experiment 1

As noted, some researchers have proposed that running-induced feeding suppression may be the result of a conditioned taste avoidance (Lett & Grant, 1996). It has been shown that consumption of a novel food that is introduced simultaneously with the wheel is almost completely suppressed for the first few weeks (Jennings & McCutcheon, 1974; Nikoletseas, 1981; Satvat & Eikelboom, 2003). According to this explanation running may make animals "sick" so that the animals suppress their food intake. If the food is novel the suppression is more severe than when the food is familiar.

If wheel running induces a direct anorexia lasting for about a day, then giving rats a novel food in combination with alternate-day wheel exposure, should induce a suppression of the novel food on wheel days and increase consumption of this food in the home cages, similar to what has been previously reported with familiar food (Mueller et al., 1997). However, if the running-induced feeding suppression is the result of a conditioned taste avoidance (Lett & Grant, 1996), then introducing rats to a novel food in combination with alternate-day wheel access should induce a suppression of the novel food on both wheel and home cage days. This explanation would imply that during

running, an association was formed between the novel food and the unconditioned effects of running, "sickness". Then suppression of the novel food should be expected not only on wheel days but also on non-wheel, home cage, days.

Therefore, the aim of the first experiment was to look at the effect of alternate-day wheel access on consumption of novel and familiar food by comparing consumption of the rats with alternate-day wheel access to that of rats with no wheel access and those with continuous wheel access.

## Method

*Subjects.* Thirty-six naïve male Sprague-Dawley rats (Charles River, Canada) weighing between 260 to 320 g at the start of the experiment were tested in two replications for this study.

*Apparatus.* Twelve standard wire cages (25 × 17 × 20 cm), each with a custom-built wheel (diameter 30 cm, width 11 cm) attached, were used for the animals with wheel access. The numbers of wheel turns were recorded using a magnetic contact closure system, in 5-s bins by the Dataquest III, a Mini-Mitter Co. data collection system. The cages were mounted on a three-tiered rack. Identical wire cages (25 × 17 × 20) were used as home cages for the animals with no wheel access and the animals in the alternate-day wheel groups when they were housed in the home cages.

*Procedure.* Upon arrival, rats were housed in pairs in standard plastic shoebox cages (48 × 27 × 20 cm) in a colony room, maintained at 21° - 22° C with a 12 hour light-dark cycle (lights on at 0700 h) for one week. During this habituation period rats had continuous access to food (Harlan Teklad 22/5 Rodent Diet) and tap water. They were weighed daily between 1000 h and 1200 h to become accustomed to handling. All the

procedures in this and all subsequent experiments were approved by the Wilfrid Laurier University Animal Care Committee following the Canadian Council of Animal Care guidelines.

Following the 7 day habituation period, rats were randomly assigned to one of three conditions, Home, Wheel, and Alternate-W. Rats were transferred from pair-housing to their assigned cages; Home group rats into the home cages; Wheel group rats into the wheels; Alternate-W group rats spent the first and consecutive odd days in the wheels and the second and consecutive even days of the experiment in the home cages. In the first replication there were 8 rats in Home group, 8 rats in Wheel group, and 4 rats in Alternate-W group. In the second replication there were 4 rats in the Home and Wheel groups and 8 rats in the Alternate-W group, resulting in 12 rats in each group.

The first four days were baseline and the wheels were locked. Food consumption was recorded daily by measuring the difference between the weight of the food when placed in the food hopper, and the weight of the food remaining (ignoring tiny crumb spillage) after 24 hours.

After the four day baseline period, wheels were unlocked and all the animals were given ad lib access to a 32% sucrose solution. Thus, each of the animals had two bottles available to them, one glass bottle filled with tap water, and a plastic bottle filled with sucrose solution. These bottles were always placed in the same position on the cage. Wheel turns, body weight, and consumptions were measured daily for 18 days. As before, food, water, and sucrose consumption were recorded daily by measuring the difference between the weight of the food, water, and sucrose before providing them to the rats, and the weight remaining after 24 hours.

*Analysis.* Food data for the baseline were analyzed using a 3 (groups) × 4 (days) mixed analysis of variance (ANOVA). For the main part of the experiment, food, sucrose, and total daily calorie intake data were analyzed separately by a 3 (groups) × 2 (conditions, odd vs. even days) × 3 (blocks of 2 days) mixed ANOVAs for the first (days 1 to 6) and last (days 13 to 18) six days of the experiment. This was followed by three planned 2 (conditions) × 3 (blocks) ANOVAs looking at each group of rats individually. The Wheel group had continuous wheel access and Alternate-W group was given access to wheels on odd days, but not on even days, thus wheel running data was analyzed by a 2 (groups) × 9 (odd days) ANOVA.

In this and subsequent experiments, for any repeated factors, results are only reported as significant if they were also significant using the Greenhouse-Geisser correction (to correct for sphericity violation). A significance level of 0.05 was used throughout.

## Results

*Wheel running:*

Figure 1 shows the number of wheel turns for the Wheel and Alternate-W groups. 2 × 9 ANOVA analysis of wheel running data found only a significant main effect of days, $F(8, 176) = 18.01$, $MSE = 1677107.06$, $p < 0.01$. This implies that there was no difference between the number of wheel turns in the two groups and that rats in both groups increased their level of running equivalently over days. Even though the rats in the Alternate-W group had access to wheel only every other day, their level of running resembles that of the rats in the Wheel group with continuous wheel access. Thus, any differences in feeding are unlikely to be due to difference in running levels.

*Familiar food consumption:*

The four baseline days of food consumption immediately prior to the unlocking of the wheel and introduction of sucrose was examined to ensure that there was no significant difference among the groups. The 3 (groups) × 4 (days) mixed ANOVA of food consumption found a significant days effect, $F(3, 99) = 26.61$, $MSE = 12.72$, $p < 0.01$ and a days × group interaction, $F(6, 99) = 2.22$, $p < 0.05$. Figure 2 shows the mean food intake of the three groups. It is evident that the rats in the three groups all increased their feeding and that this pattern across days was not consistent for each group. However, no systematic difference was found in food consumption among the three groups and the interaction appears to reflect a difference in the consumption in the three groups only on day -4. This may be the result of re-housing the rats from plastic cages (where they were paired housed) to wire cages (where they were singled housed).

The food consumption data for the first six days of the experiment were analysed in a 3 × 2 × 3 ANOVA, and showed a significant main effect of group, $F(2,33) = 30.67$, $MSE = 57.96$, $p < 0.01$, and condition, $F(1, 33) = 19.07$, $MSE = 17.37$, $p < 0.001$. The block × group, $F(4, 66) = 4.66$, $MSE = 11.07$, $p < 0.01$, condition × group, $F(2, 33) = 16.015$, $p < 0.01$, and block × condition × group interactions, $F(4, 66) = 3.91$, $MSE = 9.68$, $p < 0.01$ were all significant. To understand the nature of the significant interactions, three separate planned, 2 (conditions) × 3 (blocks) ANOVAs were performed for each group for the first 6 days of the experiment.

The ANOVA of the Home group rats revealed no significant main effects or interactions, indicating that their food intake did not change over the first 6 days of the experiment. As is evident from Figure 2, the food intake of the rats in the Home group

dropped from baseline and stayed low. This is not surprising as these rats were consuming sucrose solution (see below) and decreased their food consumption in favour of the more palatable sucrose solution.

ANOVA analysis of the Wheel group rats found only a significant effect of block, $F(2, 22) = 7.18$, $MSE = 14.15$, $p < 0.01$. As evident in Figure 2, the rats in the Wheel group decreased (relative to baseline), and then increased their food consumption over the first 6 days of the experiment. This was expected as continuous wheel access has been shown to induce an initial feeding suppression that is gone by about 7 to 10 days (e.g. Afonso & Eikelboom, 2003).

The ANOVA of the Alternate-W group rats revealed a significant effect of condition $F(1,11) = 22.33$, $MSE = 39.53$, $p < 0.01$, and a significant block × condition interaction, $F(2, 22) = 9.1$, $MSE = 8.24$, $p < 0.01$. This replicated the results reported previously by Mueller et al. (1997). When rats are given alternate-day wheel access, familiar food consumption is suppressed on wheel days and is increased on non-wheel, home cage days. This effect becomes more pronounced over the initial few blocks.

Food consumption for the last six days of the experiment was also analysed, again by a 3 (groups) × 2 (conditions) × 3 (blocks) mixed ANOVA, and showed significant main effects of group, $F(2,33) = 14.64$, $MSE = 220.55$, $p < 0.01$, block, $F(2, 66) = 12.5$, $MSE = 16.1$, $p < 0.01$, and a significant condition × group interaction, $F(2,33) = 11.83$, $MSE = 31.29$, $p < 0.01$. The results of the three planned 2 × 3 ANOVAs, one for each group for the last 6 days of the experiment, are as follows:

For the Home group rats, the ANOVA revealed a significant effect of block, $F(2, 22) = 21.16$, $MSE = 2.36$, $p < 0.01$, and a significant block × condition interaction, $F(2,$

22) = 11.14, $MSE$ = 4.99, $p < 0.01$, which is reflective of the day to day variability in feeding. As shown in Figure 2, the rats in this group changed their food consumption over the last 6 days of the experiment, consuming less rat chow possibly because they were consuming the more palatable sucrose solution (see below).

For the Wheel group rats, the ANOVA found no significant main effects or interaction over these last 6 days.

The Alternate-W group ANOVA revealed both a significant block effect, $F(2, 22) = 3.74$, $MSE = 11.76$, $p < 0.05$, and a significant condition effect, $F(1,11) = 12.03$, $MSE = 61.23$, $p < 0.01$, indicating that there was still a significant difference in consumption of the familiar rat chow between odd days and even days over the last 6 days of the experiment. As is evident in Figure 2, the Alternate-W group showed a zigzag pattern of familiar food consumption throughout the experiment. Their food consumption was suppressed on odd (wheel days), and increased on even (non-wheel, home cage) days.

*Novel Sucrose consumption:*

Sucrose consumption for the first six days of the experiment was analysed, and showed a significant main effect of group, $F(2,33) = 149.56$, $MSE = 560.39$, $p < 0.01$, and a significant block × condition interaction, $F(2, 66) = 6.57$, $MSE = 77.53$, $< 0.05$. Figure 3 shows the mean sucrose intake of the three groups of rats for the entire study. It is clear that rats without wheel access consumed significantly more sucrose in the first 6 days of the experiment than did rats in the other two groups, and that the drop in sucrose consumption in the first few days for the rats in the two wheel groups may have resulted in this significant interaction.

The subsequent planned ANOVA for the Home group rats revealed no significant effects. As is shown in Figure 3, rats in Home group consumed a lot of sucrose solution both on odd (days 1, 3, and 5) and even (days 2, 4, and 6) days.

The ANOVA of the Wheel group rats revealed a significant effect of condition $F(1,11) = 7.54$, $MSE = 49.11$, $p < 0.05$, and a significant block × condition interaction, $F(2, 22) = 9.73$, $MSE = 63.41$, $p < 0.01$. The rats in the Wheel group consumed an average of 20.27 g (± SEM = 5.36) of sucrose on the first day of the experiment and their mean sucrose consumption decreased to 4.07 g (± SEM = .84) by the second day and stayed low for day 3 to day 6. This suppression of consumption from day 1 to day 2 is most likely responsible for both significant effects.

The ANOVA of the Alternate-W group rats revealed no significant effects, as shown in Figure 3. Rats in the Alternate-W group suppressed their sucrose consumption on both odd and even days.

Sucrose consumption for the last six days of the experiment was also examined, and showed significant main effects of group, $F(2,33) = 12.58$, $MSE = 4415.6$, $p < 0.01$, block, $F(2, 66) = 7.23$, $MSE = 118.62$, $p < 0.01$, and condition, $F(1, 33) = 12.92$, $MSE = 257.97$, $p = 0.01$. Significant condition × group, $F(2,33) = 3.94$, $p < 0.05$, and block × condition interactions, $F(2, 66) = 3.49$, $MSE = 127.26$, $p < 0.05$ were also found. As Figure 3 demonstrates, rats without wheel access were still consuming significantly more sucrose at the end of the experiment than did rats in the other two groups. Based on the significant effect of block and the interaction, it appeared the three groups were increasing their consumption in the last 6 days of the study.

The planned subsequent ANOVA for each group revealed a significant block × condition interaction, $F(2, 22) = 5.73$, $MSE = 40.44$, $p < 0.05$ for the Home group, indicating that the sucrose consumption of the rats in the home cage changed over the last 6 days of the experiment. The ANOVA of the Wheel group rats found no significant main effects or interaction for the last 6 days, suggesting that consumption of the rats in this group did not change significantly. The Alternate-W group ANOVA only revealed a significant condition effect, $F(1,11) = 7.59$, $MSE = 645.51$, $p < 0.05$, indicating that there was a difference in consumption of sucrose between odd (wheel) days and even (non-wheel, home cage) days for the last 6 days of the experiment. As is apparent from Figure 3, the Alternate-W group showed a zigzag pattern of sucrose consumption over the last 6 days (day 13 to day 18). The sucrose consumption was suppressed on odd (wheel) days, and increased on even (non-wheel, home cage) days.

Similar results were found when looking at the sucrose preference ratio (sucrose consumed / sucrose + water consumed).

*Total daily energy (calorie) intake:*

Since rats had two sources of calories, sucrose solution and chow, it was also reasonable to investigate their total daily energy intake. Total calorie intake was calculated by adding up energy intake from both rat chow (metabolizable energy = 2.98 Kcal/g) and 32% sucrose solution (metabolizable energy = 1.28 Kcal/g). Figure 4 shows total daily calorie intake of the three groups over the entire study. Note that baseline calorie data is the same as the chow data as this was the only source of calories animals had at this time.

The first six days of total calorie intake data was analyzed by a 3 × 2 × 3 ANOVA, and demonstrated a significant main effect of group, $F(2,33) = 78.77$, $MSE = 772.98$, $p < 0.01$, and condition, $F(1, 33) = 9.03$, $MSE = 356.22$, $p < 0.001$. The block × group, $F(4, 66) = 2.63$, $MSE = 134.6$, $p < 0.05$, condition × group , $F(2, 33) = 13.3$, $p < 0.01$, block × condition, $F(2, 66) = 12.57$, $MSE = 160.92$, $p < 0.01$, and the three way block × condition × group interactions, $F(4, 66) = 4.26$, $p < 0.05$ were all significant. Results of the subsequent three planned 2 (conditions) × 3 (blocks) ANOVAs, one for each group for the first 6 days of the experiment, are as follows:

The ANOVA of the Home group rats revealed no significant main effects or interactions, indicating that total energy intake of the rats in this group did not change over the first 6 days of the experiment. From Figure 4, it is clear that relative to baseline the total calorie intake of the rats in the Home group was elevated on the first day of sucrose availability and stayed high. This is not surprising as these rats were consuming a large amount of highly preferred sucrose solution in addition to their chow.

The ANOVA of the Wheel group rats found significant effects of condition, $F(1, 11) = 5.43$, $MSE = 122.57$, $p < 0.05$, and block × condition, $F(2, 22) = 13.31$, $MSE = 137.86$, $p < 0.01$. As evident in Figure 4 the rats in the Wheel group decreased and then increased their total energy intake over the first 6 days of the experiment.

The Alternate-W group ANOVA revealed significant effects of block, $F(2, 22) = 5.15$, $MSE = 80.23$, $p < 0.05$, condition $F(1,11) = 2.13$, $MSE = 469.1$, $p < 0.01$, and block × condition, $F(2, 22) = 11.26$, $MSE = 128.67$, $p < 0.01$. As shown in Figure 4, the rats in the Alternate-W group decreased their total calorie intake on day 1, 3, and 5 when they had wheel access and increased their energy intake on even days when they were

back in their home cages. This replicates the results reported previously by Mueller et al. (1997), when rats are given alternate-day wheel access, food consumption (hence energy intake) is suppressed on wheel days and is higher on non-wheel, home cage, days, but still not as high as the Home cage animals' consumption.

Total energy intake for the last six days of the experiment was analysed similarly for all three groups of rats, and showed significant main effects of group, $F(2,33) = 10.05$, $MSE = 2971.11$, $p < 0.01$, block, $F(2, 66) = 7.22$, $MSE = 145.42$, $p < 0.05$, and condition, $F(1, 33) = 30.66$, $MSE = 343.95$, $p < 0.01$. The condition × group interaction, $F(2,33) = 27.27$, $p < 0.01$ was also significant. Three planned ANOVAs were performed for each group for the last 6 days of the experiment to understand the nature of significant interaction.

For both the Home and Wheel group rats, separate 2 × 3 ANOVAs revealed no significant main effects or interactions, indicating that total energy intake of the rats in both of these groups were stable over the last 6 days of the experiment. However, the Alternate-W group ANOVA revealed both a significant block effect, $F(2, 22) = 4.71$, $MSE = 167.96$, $p < 0.05$, and a significant condition effect, $F(1,11) = 142.96$, $MSE = 676.46$, $p < 0.01$, indicating that, similar to the first six days of the experiment, there was still a significant difference in total energy intake between odd (wheel) days and even (non-wheel, home cage) days. As is evident in Figure 4, the Alternate-W group maintained a zigzag pattern of energy intake throughout the experiment.

*Last day body weight:*

A one-way ANOVA on the body weights of the three groups of rats for the last day of the study (day 18) was performed to look at the cumulative effect of consumption

and exercise on body weight. There was a significant group effect, $F(2, 33) = 11.12$, $MSE = 859.94$, $p < 0.001$. The LSD post hoc revealed that the body weights of the three groups were all different. On the last day of the study, the Home group rats weighed 431.21 ± 7.9 g, the Wheel group rats 406.71 ± 10.26 g, and the Alternate-W group rats 374.92 ± 6.14 g. The difference in the weights of the three groups possibly reflects the difference in both the food and sucrose consumption, and in energy expenditure over the combined effects of both wheel and non-wheel days.

## Discussion

Taken together, the animals in the home cage group consumed significantly more novel sucrose from day 1, and maintained this consumption throughout the study, relative to the other two groups. Even though the rats in this group suppressed their intake of familiar rat chow in favour of more palatable novel sucrose, their total daily calorie intake increased on day 1 and was maintained at this level throughout the study.

The running of rats in the two groups with wheel access increased at the same rate and therefore, any differences in food intake are unlikely to be due to differences in running. Sucrose consumption of the rats with continuous wheel access was almost completely suppressed initially, and then increased gradually towards the end of the study. The intake of the familiar rat chow was also suppressed; however, consumption of familiar rat chow recovered more rapidly than the consumption of novel sucrose. The intake suppression of both chow and sucrose in this group of rats is reflected in their total daily calorie intake. When the wheel was introduced to these animals, their total daily calorie intake was suppressed at first but by the end of the study the total calorie intake of these rats had increased and became similar to that of the home cage control group. These

results are consistent with previous work, looking at the effects of wheel introduction on feeding (Levitsky, 1970; Looy & Eikelboom, 1989; Premack & Premack, 1963). Wheel introduction has a much stronger and long lasting suppressive effect on intake of a novel compared to a familiar food (Satvat & Eikelboom, 2003).

The group of rats with alternate-day wheel exposure showed a zigzag pattern of familiar food intake from day 1: suppressing their intake of the familiar rat chow on wheel days and increasing their intake of the chow on the intervening non-wheel days. These animals suppressed their intake of the novel sucrose at the start of the experiment on both the home cage and wheel days. By the end of the study for the last 6 days, however, they consumed more sucrose when they were in their home cage compared to when they had access to the wheel, and showed a zigzag pattern of sucrose consumption.

In sum, the results from Experiment 1 revealed that rats with alternate-day wheel exposure suppressed their consumption of familiar food on wheel days and increased their consumption on non-wheel, home cage days, throughout the study. However, the same animals initially suppressed their consumption of the novel sucrose solution on all days. These results suggest that alternate-day wheel exposure has different initial effects on novel than on familiar food. The results are consistent with a role for conditioned taste avoidance in the novel food suppression. During the first day, novel food is paired with unconditioned effect of wheel running, which according to Lett and Grant (1996) is "sickness", thus the animals associate the "sickness" with the novel food. The animals then avoid consuming the novel food in their home cages due to the conditioned association with wheel running. This is not the case for the familiar food. Only on the first and consecutive odd days do the rats suppress their intake of the familiar food

because of the unconditioned effect of running, which directly influences intake. On even days when the rats are in their home cages they consume familiar food normally.

## Experiment 2

CS pre-exposure has been shown to reduce a conditioned taste avoidance, a phenomenon termed latent inhibition (Lubow & Moore, 1959; Lubow, 1989). If latent inhibition interferes with the association between sucrose and the physiological effects induced by wheel running, then sucrose pre-exposure should reduce the learned sucrose suppression. If the group of rats with alternate-day wheel exposure is given sucrose pre-exposure, then these rats should fail to make an association between the now-familiar sucrose solution and the unconditioned effect of running with wheel introduction. The rats would still experience the unconditioned effect of wheel running and so would reduce their familiar sucrose intake when wheels are available, but they would not associate the unconditioned effect of running with familiar sucrose and should not show a conditioned suppression on non-wheel days. In other words, on wheel days, familiar sucrose consumption would be suppressed due to the unconditioned effect of running, however on non-wheel days, sucrose consumption should be elevated because no association has been made between the unconditioned effect of running and the familiar sucrose solution.

Experiment 1 demonstrated that the running-induced feeding suppression may be the result of conditioned taste avoidance, because introducing rats to a novel food, in combination with alternate-day wheel access, suppressed the novel food consumption on both wheel and non-wheel days. During running, an association between the novel food

and the unconditioned effect of running may have been formed, so that the animals suppressed their intake of the novel food not only on wheel days but also on non-wheel, home cage, days. Such an association did not form with the familiar rat chow. Thus, if the novel sucrose solution were to be made familiar by sucrose pre-exposure, the association between the unconditioned effect of wheel running and the sucrose solution would not be formed, and the zigzag pattern of sucrose consumption would be expected earlier in the study.

The aim of the Experiment 2 was to make the novel sucrose familiar before initiating alternate-day wheel exposure. It was hypothesized that the consumption of now-familiar sucrose would be suppressed on wheel days and be increased on non-wheel, home cage days in rats with alternate-day wheel exposure.

## Method

*Subjects.* Twenty-four naïve male adult Sprague-Dawley rats (Charles River, Canada) weighing between 270 to 350 g at the start of the experiment, were used for this study.

*Apparatus.* The cages and wheels used in this experiment were the same as those of Experiment 1.

*Procedure.* Upon arrival, the rats were housed individually in hanging wire cages (25 × 17 × 20 cm), and were handled and weighed daily. Rats had free access to food (Harlan Teklad 22/5 Rodent Diet) and tap water. Three days after arrival, 32% sucrose solution was offered to all the rats. Animals had two bottles available to them, one bottle filled with tap water and the other one filled with sucrose solution. Ad lib food was also available in the food hopper.

After four days of sucrose exposure, 6 days of baseline were started. The rats were randomly assigned to one of three conditions, Home, Wheel, and Alternate-W. The 8 rats in the Home group remained in their wire cages. The 8 rats in the Wheel group were transferred into the wire cages, attached to wheels, with the wheels locked. The 8 rats in the Alternate-W group started the first day and consecutive odd days in the locked wheel cages; and on the second day and consecutive even days were in the hanging wire home cages. Body weight, sucrose, food, and water intakes were recorded daily for the six days of baseline. Consumption was recorded by measuring the difference between the weight of the food, sucrose or water when placed in the cage, and the weight remaining after 24 hours.

After 10 days of sucrose pre-exposure (4 days of habituation and 6 days of baseline), wheels were unlocked. With only 12 wheels, 4 of the wheels were shared by Alternate-W group rats. Four of the rats in this group that were lightest in weight, at the start of habituation, started the experiment one day later than the other animals. All the rats were provided with ad lib food and bottles of water and bottles of 32% sucrose solution for 18 days. Body weight, food, water and sucrose consumption of each rat was measured as before.

*Analysis.* For the baseline (day -5 to 0), first (days 1 to 6) and last (days 13 to 18) six days of the experiment, food, sucrose, and total calorie intake data were analyzed separately using a 3 (groups) × 2 (conditions, odd vs. even days) × 3 (blocks of 2 days) mixed ANOVAs. This was followed by three planned 2 (conditions) × 3 (blocks) ANOVAs looking at each group separately.

The rats in the Wheel group had continuous access to wheels but the Alternate-W group rats were given access to wheels only on odd days, therefore wheel running data was analyzed using a 2 (groups: Wheel vs. Alternate-W) × 9 (odd days) ANOVA.

## Results

*Wheel running:*

Wheel running data was analyzed by a 2 × 9 ANOVA and only a significant main effect of days, $F(8, 112) = 10.44$, $MSE = 3106088.1$, $p < 0.01$ was found. This demonstrates, as is evident in Figure 5, that there was no difference in wheel running between the two groups and that the rats in both groups increased their running similarly over days.

*Food consumption:*

In order to be assured that there was no significant difference among the groups in food consumption before the wheels were unlocked, the six baseline days of food consumption immediately prior to the introduction of wheel were studied. The 3 × 2 × 3 ANOVA of food consumption revealed only a significant block effect, $F(2, 42) = 15.55$, $MSE = 4.76$, $p < 0.01$. From Figure 6, which shows the mean food consumption of the three groups, it is evident that the food consumption of the rats in the three groups varied from day to day over these six days of baseline.

The food consumption data for the first six days of the experiment were examined, and revealed a significant main effect of block, $F(2,42) = 3.7$, $MSE = 3.93$, $p < 0.05$, and condition, $F(1, 21) = 16.96$, $MSE = 13.44$, $p < 0.01$. The block × group, $F(4, 42) = 3.47$, $p < 0.05$, condition × group, $F(2, 21) = 13.47$, $p < 0.01$, and block × condition interactions, $F(2, 42) = 6.82$, $MSE = 9.68$, $p < 0.05$ were all significant. Thus,

three separate planned 2 (conditions) × 3 (blocks) ANOVAs were performed for each group for the first 6 days of the experiment.

The ANOVA of the Home group rats found a significant main effect of block, $F(2,14) = 7.31$, $MSE = 0.66$, $p < 0.05$, and it appears from Figure 6 that rats in the Home group decreased their food intake over this period. The rats in the home cage consumed most of their daily energy intake from the more palatable sucrose solution (see below).

The ANOVA of the Wheel group rats found significant effects of block, $F(2, 14) = 7.02$, $MSE = 5.22$, $p < 0.05$, and condition, $F(1, 7) = 7.4$, $MSE = 51.99$, $p < 0.05$. As evident in Figure 6, the rats in the Wheel group decreased and then increased their food consumption over the first 6 days of the experiment.

The ANOVA of the Alternate-W group revealed a significant effect of condition $F(1,7) = 15.76$, $MSE = 36.35$, $p < 0.05$, and a significant block × condition interaction, $F(2, 14) = 6.92$, $MSE = 3.01$, $p < 0.05$. When rats were given alternate-day wheel access, familiar food consumption was suppressed on wheel days and was increased on non-wheel, home cage, days. Figure 6 represents this zigzag pattern of food consumption in the Alternate-W group. This replicated the results of Experiment 1 and the results reported previously by Mueller et al. (1997).

Food consumption for the last six days of the experiment were also analysed using a 3 × 2 × 3 mixed ANOVA, and a significant main effect of block, $F(2,42) = 8.43$, $MSE = 3.02$, $p < 0.05$, a condition × group, $F(2,21) = 3.9$, $MSE = 11.44$, $p < 0.05$, and block × condition interactions, $F(2, 42) = 4.53$, $MSE = 4.38$, $p < 0.05$ were found. The results of the three planned 2 (conditions) × 3 (blocks) ANOVAs, for each group for the last 6 days of the experiment, were as follow:

For the Home group rats, the ANOVA found neither significant main effects nor interactions over day 13 to 18, indicating that the food consumption of the rats in the Home group had stabilized and did not change. For the Wheel group rats, the ANOVA revealed only a significant effect of block, $F(2, 14) = 8.28$, $MSE = 2.52$, $p < 0.05$. It appears from Figure 6 that the rats in this group decreased their food consumption over the last 6 days, perhaps in favour of the more palatable sucrose solution. The Alternate-W group ANOVA revealed neither significant main effects nor interactions over the last 6 days, indicating that food intake of the rats in this group stayed the same on odd (wheel) days and even (non-wheel, home cage) days.

*Familiar sucrose consumption:*

Rats in all groups had 10 days of pre-exposure to sucrose. The six days (baseline) of sucrose intake data immediately before the introduction of the wheel was analyzed to ensure that there were no significant differences in sucrose intake prior to the start of the experiment. The 3 × 2 × 3 ANOVA of sucrose intake data for the baseline found significant effects of block, $F(2, 42) = 24.44$, $MSE = 28.12$, $p < 0.01$, and condition, $F(1, 21) = 4.7$, $MSE = 72.05$, $p < 0.05$, and a significant block × condition interaction, $F(2, 42) = 3.45$, $MSE = 24.12$, $p < 0.05$. These results indicate that the rats in the three groups, as shown in Figure 7, all increased their sucrose intake over the baseline period, but that the groups did not differ.

The data describing sucrose consumption for first six days of the experiment were analysed, and showed a significant main effect of group, $F(2, 21) = 5.21$, $MSE = 1274.25$, $p < 0.05$, block, $F(2,42) = 4.34$, $MSE = 77.29$, $p < 0.05$, and condition, $F(1, 21) = 10.16$, $MSE = 115.05$, $p < 0.05$. The block × group, $F(4, 42) = 4.19$, $p < 0.05$, condition

× group, $F(2, 21) = 10.16, p < 0.05$, and block × condition interactions, $F(2, 42) = 5.61$, $MSE = 82.39, p < 0.05$ were all significant. Thus, there was a significant difference in sucrose consumption of the three groups for the first six days of the experiment. The results of the three separate planned 2 × 3 ANOVAs for each group, for the first 6 days of the experiment, were as follows:

The planned subsequent ANOVA for the Home group rats revealed only a significant effect of block, $F(2, 14) = 4.4$, $MSE = 748.8$, $p < 0.05$. As shown in Figure 7, rats in Home group increased their sucrose intake over these 6 days.

The ANOVA of the Wheel group rats revealed a significant effect of block, $F(2, 14) = 4.76$, $MSE = 98.8$, $p < 0.05$, and a significant block × condition interaction, $F(2, 14) = 5.5$, $MSE = 42.08$, $p < 0.05$. The rats in the Wheel group consumed an average of 66.1 ± 7.6 (SEM) g of sucrose on day 1and their mean sucrose consumption decreased to 50.2 ± 7.9 g by the day 3 and increased to 62.1 ± 7.0 g for day 6. As is evident from Figure 7, the initial suppression of sucrose consumption recovers by day 6 and this change in consumption was responsible for both significant effects. This was consistent with previous studies reporting introduction of running wheel suppressed food intake (e.g. Afonso & Eikelboom, 2003).

The ANOVA of the Alternate-W group rats revealed a significant effect of condition, $F(1, 7) = 13.54$, $MSE = 246.29$, $p < 0.05$, and a significant block × condition interaction, $F(2, 14) = 4.37$, $MSE = 112.39$, $p < 0.05$, indicating that there was a difference in consumption of sucrose on odd (wheel) days and even (non-wheel, home cage) days over these 6 days. As evident from Figure 7, the Alternate-W group showed a

zigzag pattern of sucrose consumption for the first 6 days (day 1 to day 6) of alternate-day wheel exposure.

Sucrose consumption for the last six days of the experiment showed a significant main effect of group, $F(2, 21) = 4.45$, $MSE = 1048.15$, $p < 0.05$, and condition, $F(1, 21) = 38.53$, $MSE = 120.47$, $p = 0.01$. A significant block × group, $F(4, 42) = 4.57$, $p < 0.05$, and condition × group interaction, $F(2, 21) = 30.02$, $MSE = 120.47$, $p < 0.01$ were also found.

The planned subsequent ANOVA of the Home group revealed significant effects of block, $F(2, 14) = 5.42$, $MSE = 25.73$, $p < 0.05$, condition, $F(1, 7) = 8.97$, $MSE = 14.55$, $p < 0.05$, and block × condition, $F(2, 14) = 5.78$, $MSE = 29.52$, $p < 0.05$. As shown in Figure 7 the rats in Home group appeared to be increasing their sucrose intake over the last 6 days of the study.

The ANOVA of the Wheel group rats found no significant main effects or interaction for the last 6 days, suggesting that sucrose consumption of the rats in this group did not change.

The Alternate-W group ANOVA only revealed a significant condition effect, $F(1, 7) = 37.03$, $MSE = 317.07$, $p < 0.01$, indicating that there was still a difference in consumption of sucrose between odd (wheel) days and even (non-wheel, home cage) days over the last 6 days of the experiment. As apparent from Figure 7, the Alternate-W group showed a zigzag pattern of sucrose consumption for the last 6 days (day 13 to day 18) with sucrose intake being much lower on wheel days.

Similar results were found by looking at sucrose preference ratio (sucrose consumed / sucrose + water consumed) (Data not shown).

*Total daily energy (calorie) intake:*

Rats in the Alternate-W group showed a zigzag pattern of sucrose intake after introduction to alternate-day wheel exposure. The same rats however, showed a zigzag pattern of food consumption only for the first period of alternate-day wheel exposure. Their food consumption on odd (wheel) days was the same as their food intake on even (non-wheel, home cage) days for the latter part of the experiment. Thus, it seemed reasonable to investigate the total daily energy intake of the rats with alternate-day wheel access in order to determine whether it followed a zigzag pattern. Total calorie intake was calculated as before (Experiment 1).

Figure 8 shows the mean total caloric intake of the three groups for the entire 18 days of the experiment. Starting with the six days of baseline, the 3 × 2 × 3 ANOVA of total calorie intake revealed a significant block effect, $F(2, 42) = 7.04$, $MSE = 69.74$, $p < 0.05$. The block × group, $F(4, 42) = 2.63$, $p < 0.05$, and block × condition, $F(2, 42) = 3.53$, $MSE = 72.64$, $p < 0.05$ interactions were also significant. Subsequent planned ANOVA for each group found only a significant effect of block, $F(2, 14) = 7.73$, $MSE = 103.45$, $p < 0.05$, for the Home group with the consumption going up, and a significant effect of block, $F(2, 14) = 4.52$, $MSE = 42.59$, $p < 0.05$, for the Alternate-W group with the consumption going up.

The first six days of total calorie intake data, after wheel introduction, was analyzed by a 3 × 2 × 3 ANOVA, and significant main effects of group, $F(2, 21) = 5.86$, $MSE = 3201.89$, $p < 0.01$, block, $F(2, 42) = 6.7$, $MSE = 167.02$, $p < 0.01$, and condition, $F(1, 21) = 17.34$, $MSE = 422.85$, $p < 0.001$ were found. The block × group, $F(4, 42) = 4.03$, $p < 0.01$, condition × group, $F(2, 21) = 16.36$, $p < 0.01$, and block × condition,

$F(2, 42) = 9.61$, $MSE = 135.39$, $p < 0.01$, interactions and the three way block × condition × group interaction, $F(4, 42) = 2.78$, $p < 0.05$ were all significant. Results of the planned 2 (conditions) × 3 (blocks) ANOVAs for each group for the first 6 days of the experiment were as follows:

The ANOVA of the Home group rats showed a significant effect of block, $F(2, 14) = 4.01$, $MSE = 72.19$, $p < 0.05$, indicating, as evident in Figure 8, that the rats in this group increased their energy intake over the first 6 days of the study. The ANOVA of the Wheel group rats revealed a significant effect of block, $F(2, 14) = 7.64$, $MSE = 209.83$, $p < 0.01$ and a significant block × condition interaction, $F(2, 14) = 6.78$, $MSE = 95.38$, $p < 0.01$. As is evident in Figure 8, the rats in the Wheel group decreased and then increased their total energy intake over the first 6 days of the experiment. Based on previous work, this was expected (e.g. Afonso & Eikelboom, 2003).

The Alternate-W group ANOVA revealed a significant effect of condition, $F(1, 7) = 20.45$, $MSE = 1031.27$, $p < 0.01$, and a significant block × condition interaction, $F(2, 14) = 7.06$, $MSE = 199.2$, $p < 0.01$. As depicted in Figure 8, the rats in the Alternate-W group decreased their total calorie intake on day 1, 3, and 5 when they had access to the wheel and maintained their total calorie intake on even days when they were in their home cages, a difference that became more pronounced as the experiment progressed.

Total energy intake over the last six days of the experiment was also analysed, and showed significant effects of block, $F(2, 42) = 5.64$, $MSE = 81.22$, $p < 0.01$, and condition, $F(1, 21) = 40.63$, $MSE = 231.69$, $p < 0.001$. The block × group, $F(4, 42) = 3.8$, $p < 0.05$, condition × group, $F(2, 21) = 40.45$, $p < 0.01$, and block × condition, $F(2, 42) = 8.47$, $MSE = 81.92$, $p < 0.01$, interactions were all significant.

For the Home group rats, the 2 × 3 ANOVA revealed a significant effect of block, $F(2, 14) = 6.3$, $MSE = 62.25$, $p < 0.05$, and a significant block × condition interaction, $F(2, 14) = 6.85$, $MSE = 95.19$. Similarly, the ANOVA of the Wheel group rats found a significant effect of block, $F(2, 14) = 8.81$, $MSE = 71.13$, $p < 0.01$, and a significant block × condition interaction, $F(2, 14) = 6.62$, $MSE = 50.9$, suggesting that total calorie consumption was not very stable in both these groups.

The Alternate-W group ANOVA revealed only a significant condition effect, $F(1, 7) = 53.98$, $MSE = 518.81$, $p < 0.01$, indicating that, similar to when wheel exposure was started, there was a significant decrease in total energy intake on odd (wheel) days compared to even (non-wheel, home cage) days. As represented in Figure 8 the Alternate-W group showed a zigzag pattern of energy intake throughout the experiment. Thus, even though only the sucrose intake and not the food intake of the rats in this group for the last 6 days of the study showed a zigzag pattern, the total calorie intake of these rats was decreased on the wheel exposure days.

*Last day body weight:*

A one-way ANOVA on the body weights of the three groups of rats for the last day of the study (day 18) revealed a significant between group effect, $F(2, 21) = 3.85$, $MSE = 2415.54$, $p < 0.05$. The LSD post hoc revealed that the body weights of Home group rats was different from that of the Alternate-W group rats. On the last day of the study, the Home group rats weighed 410.69 ± 25.6 g, the Wheel group rats 378.38 ± 14.7 g, and the Alternate-W group rats 342.50 ± 5.8. The difference in body weights may be due to differences in total calorie intake of rats in these groups.

## Discussion

Although the rats with the alternate-day wheel exposure had wheel access only every other day, their running level increased at the same rate as that of the rats with continuous wheel exposure. Thus, differences in food intake of these two groups are unlikely to be due to differences in running levels.

Unlike Experiment 1, the rats with continuous wheel access suppressed their consumption of both the familiar rat chow and the familiar sucrose solution for only a short period and their consumption recovered after only a few days. In Experiment 1, introduction of the wheel completely suppressed intake of the novel sucrose. This experiment demonstrates that sucrose pre-exposure considerably reduced the wheel-induced suppression of sucrose solution.

The group of rats with alternate-day wheel exposure showed a zigzag pattern of familiar sucrose intake from day 1 of wheel exposure lasting throughout the entire study: suppressing intake of the sucrose solution on wheel days and increasing their consumption of the sucrose solution on the intervening non-wheel days. This agrees with our hypothesis that the effect of alternate-day wheel exposure on novel food is different from its effect on familiar food. These animals showed a similar alternate-day pattern of rat chow intake at the beginning of the experiment; however, toward the end of the study rat chow consumption had stabilized. Rats were consuming the same amount of rat chow regardless of wheel accessibility. Thus, toward the end of the study, rats were only suppressing their intake of the sucrose solution and not the rat chow on wheel days. On non-wheel, home cage days the rats were consuming the same amount of rat chow as the day before, but increasing their sucrose consumption considerably. This is not clear

whether this difference in consumption is due to a difference in familiarity or a difference in preference. Looking at the total daily calorie intake revealed that for these rats caloric intake followed the zigzag pattern throughout the study.

## Experiment 3

### *The Effect of Age on Running Induced-Suppression of a Novel Food*

Similar to adult rats, weanlings (rats younger than 35 days of age) run instinctively when introduced to running wheels. When 25 days old weanlings are introduced to running wheels, they increase their running over days and by 50 days of age their running reaches a plateau (Cortright, Chandler, Lemon, & DiCarlo, 1997; Pitts, 1984). Afonso (2000) demonstrated that, despite the large weight difference between adult and weanling male rats, their patterns of wheel running were similar. Both adult and weanling rat's running increased at the same rate and reached a plateau at the same level.

Interestingly, wheel running appears to have no effect on food consumption of weanlings (Afonso, 2000; Pitts, 1984). Afonso (2000) compared food consumption of adult and weanling male rats. Even though the weanling's pattern of running was observed to be similar to that of adults, the effect of running on food consumption appeared to be different. Unlike the wheel's suppressive effect on feeding in adults, wheel running did not induce a feeding suppression in weanlings. Francis (2002) investigated the effects of running on food consumption of female weanling rats. Again no suppression of food consumption was evident in the female weanling rats at wheel introduction. These results suggest that there is an age difference in the action of wheel running on food consumption.

Both female and male weanlings are experiencing a period of rapid growth, during which they consume large amounts of food and gain weight rapidly compared to adult rats. The peak period of weight gain for male rats is between 28 and 49 days of age (Leibowitz, Lucas, Leibowitz, & Jhanwar, 1991). Furthermore, whereas weanlings are adding lean body mass, adult rats are adding fat. Thus, it has been suggested that (Afonso, 2000), when the introduction of the wheel coincides with this rapid growth period, no effect on feeding occurs.

These data suggest that wheel running does not induce a feeding suppression of familiar foods in weanlings. Weanlings are exposed to the rat chow from birth and start consuming the food (Purina rat chow) even before weaning. In adult rats, wheel running induces a much stronger suppression of a novel than of a familiar food (Satvat & Eikelboom, 2003). A start period of wheel running also induces a conditioned taste avoidance to a novel taste in adult rats (Hayashi et al., 2002; Heth et al., 2001; Lett & Grant, 1996; Lett et al. 1998; Lett et al. 2001; Nakajima et al., 2000; Salvy et al., 2002). Considering conditioned taste avoidance data and that wheel running does not induce a suppression of a familiar food in weanlings, this question occurs: Does wheel running induce a suppression of a novel food in weanlings? Thus, the aim of the Experiment 3 was to determine the effects of wheel access on novel food consumption in weanlings, comparing it with that of adults.

## Method

*Subjects.* Thirty-two weanling (21- 23 days old), and thirty-two adult (47 – 49 days old) male Sprague-Dawley rats, were used for this study. Upon arrival, the rats were individually housed in polycarbonate shoebox cages (46 × 24 × 15 cm) for weanlings

and (45 × 25 × 20 cm) for adults, in a colony room, maintained at 21° - 22° C with a 12 hours light-dark cycle (lights on at 0600 h). The animals had continuous access to food (Harlan Teklad 22/5 Rodent Diet) and tap water, throughout the study. Because of equipment limitations this experiment was run in four replications.

*Apparatus.* Sixteen polycarbonate shoebox cages (45× 24 × 20 cm), each with a commercially-built Nalgene wheel (diameter 30 cm, width 11 cm) in the cage were used. The numbers of wheel turns were recorded using a magnetic contact closure system, in 5-s bins by the Vital View data collection system from Mini-Mitter Co.

*Procedure.* Upon arrival, all rats were housed individually in the shoebox cages for one week so that they would habituate to the laboratory conditions. During habituation, the rats were weighed daily between 0900 h and 1100 h to become accustomed to handling. Following the habituation period, the rats were assigned to one of four conditions, Sucrose-Wheel, No Sucrose-Wheel, Sucrose-Locked Wheel, and No Sucrose-Locked Wheel. The last three days of habituation were a baseline period, during which the food and water intakes were measured.

After the 7 day habituation, all the rats were transferred to plastic cages equipped with Nalgene wheels. The wheels of half of the rats were locked. Rats assigned to get sucrose were given ad lib access to a 32% sucrose solution. These rats had one bottle of tap water and one bottle of sucrose solution, whereas the rats with no sucrose access had two bottles of tap water available to them. Sucrose, water, and food intake were measured daily as the difference between the weight of the food, water, and sucrose offered to the animals, and remaining weight after 24 hours. Consumptions, body weight, and running data were collected throughout the experiment for the total of 10 days.

*Analysis.* For the baseline, the food data were analyzed using a 2 (age: adults vs. weanlings) × 2 (wheel vs. locked wheel) × 2 (sucrose vs. no sucrose) × 3 (days) mixed analysis of variance (ANOVA).

For the main part of the experiment, the food, and the total daily calorie intake for each age were analyzed separately by 2 (sucrose vs. no sucrose) × 2 (wheel vs. locked wheel) × 5 (days) mixed ANOVAs for the first (day 1 to 5) and last (day 6 to 10) five days of the study. The sucrose intake data for the groups with sucrose access were also analyzed by 2 (age) × 2 (wheel) × 5 (days) mixed ANOVAs for the first and last five days of the experiment. The wheel running data were analyzed in two five-day blocks for the first and last five days of the experiment, using 2 (age) × 2 (sucrose) × 5 (days) ANOVAs.

## Results

There were 5 incidents of sucrose spillage (wet bedding under the sucrose bottle) for one of the adult rats in the Sucrose-Wheel condition, thus all the data for this rat were removed from the analyses.

*Wheel running:*

The 2 × 2 × 5 ANOVA of the first 5 days of wheel running revealed a significant effect of days, $F(4, 108) = 6.83$, $MSE = 134774.48$, $p < 0.01$, and a significant days × age interaction, $F(4, 108) = 3.56$, $p < 0.01$. This was followed by planned comparison looking at each age group separately. The 2 (group) × 5 (day) ANOVA revealed non-significant days effect for weanlings. However, there was a significant days effect for adults, $F(4, 52) = 12.07$, $MSE = 242197.76$, $p < 0.01$. Figure 9 shows the wheel running data for both adults and weanlings. It can be seen that there is no marked age difference in the amount

of running over the first 5 days. However, the days × age interaction implies that the escalation of running was different for adults and weanlings. The planned comparison revealed that adult rats, but not weanlings, increased their level of running.

Similar planned ANOVA for the last five days of the study found significant effects of age, $F(1, 27) = 15.8$, $MSE = 14002042.56$, $p < 0.01$, days, $F(4, 108) = 6.24$, $MSE = 1256335.06$, $p < 0.01$, and a significant days × age interaction, $F(4, 108) = 7.87$, $p < 0.01$. A subsequent planned comparison found no days effect for weanlings, indicating that their running remained stable, but there was a significant days effect for adults, $F(4, 52) = 7.97$, $MSE = 2069246.18$, $p < 0.01$. As is shown in Figure 9, both groups of adults were running considerably more than the two groups of weanlings for the last five days of study. Adults continued to increase their level of running over these last five days.

In addition, sucrose and no sucrose groups did not differ in running at any time. This suggests that sucrose availability did not influence the rat's wheel running.

*Baseline food intake:*

The three days of food intake data immediately prior to the introduction of wheel and sucrose were examined using 2 (age) × 2 (wheel) × 2 (sucrose) × 3 (days) mixed analysis ANOVA, which revealed a significant main effect of age, $F(1, 55) = 1217.78$, $MSE = 4.2$, $p < 0.01$, and a significant days effect, $F(2, 110) = 5.88$, $MSE = 3.09$, $p < 0.01$. These results indicate that adult rats were consuming significantly more food than the weanlings. The four groups of adults and the four groups of weanlings also showed fluctuations in their food intake over the 3 days of baseline. Figure (10-A) and (10-B) show the food intake of adults and weanlings respectively. As is evident for each age, the four groups of rats consumed similar amount of food during the baseline.

Because of expected age difference in food consumption, it was decided to analyse food and total daily calorie intakes of adults and weanlings separately.

*Food intake of adults:*

The food consumption of adults was analyzed using 2 (sucrose) × 2 (wheel) × 5 (days) mixed ANOVAs. For the first five days, the ANOVA found a significant main effect of wheel, $F(1, 27) = 5.847.02$, $MSE = 26.27$, $p < 0.05$, sucrose, $F(1, 27) = 18.71$, $p < 0.01$, and days, $F(4, 108) = 30.21$, $MSE = 8.69$, $p < 0.01$. The wheel × sucrose, $F(1, 27) = 40.18$, $p < 0.01$, and days × wheel interactions, $F(4, 108) = 14.43$, $p < 0.01$ were also significant. This analysis was followed by planned comparison using 2 (wheel) × 5 (days) ANOVAs to look separately at the groups with and without sucrose access.

For the groups with no sucrose access there were significant effects of wheel, $F(1, 14) = 67.29$, $MSE = 137.6$, $p < 0.01$, and days $F(4, 56) = 11.16$, $MSE = 12.2$, $p < 0.01$ and a significant days × wheel interaction, $F(4, 56) = 7.14$, $p < 0.01$. Figure (10-A) shows the food consumption of adults. It is evident that the rats in No Sucrose-Locked Wheel group showed relatively stable food intake but the rats in No Sucrose-Wheel group suppressed their food intake initially and then increased it over the first five days of the study.

A similar planned ANOVA for the groups with sucrose access found a significant wheel effect $F(1, 13) = 5.16$, $MSE = 37.87$, $p < 0.05$, days effect $(4, 52) = 26.98$, $MSE = 4.92$, $p < 0.01$, and days × wheel interaction, $F(4, 52) = 9.95$, $p < 0.01$. As is shown in Figure (10-A), there was a suppression of food intake in both groups of rats given sucrose access relative to no wheel no sucrose control rats. The rats in Sucrose-Wheel group then increased their food intake over the first five days of the study, much like the rats in the

No Sucrose-Wheel group. The rats in Sucrose-Locked Wheel group did not increase their intake, perhaps because the animals in this group were consuming a large amount of sucrose (see below- sucrose intake and total calorie intake).

A 2 × 2 × 5 ANOVA of the food intake for the last 5 days of the experiment, found a significant main effect of sucrose, $F(1, 27) = 30.29$, $MSE = 55.66$, $p < 0.0$, and days, $F(4, 108) = 4.09$, $MSE = 6.01$, $p < 0.01$. The wheel × sucrose, $F(1, 27) = 13.73$, $p < 0.01$, and days × sucrose interactions, $F(4, 108) = 4.31$, $p < 0.01$ were also significant. This was followed by two planned 2 (wheel) × 5 (days) ANOVAs to look at groups with and without sucrose access separately.

For the groups with no sucrose access there was only a significant days effect $F(4, 56) = 4.06$, $MSE = 7.51$, $p < 0.01$. As shown in Figure (10-A), both groups of rats with no sucrose showed changes in their food intake over the last five days of the study but wheel access did not influence food intake of the rats at this time.

Similar planned ANOVA for the groups with sucrose access found a significant wheel, $F(1, 13) = 13$, $MSE = 64.74$, $p < 0.01$, and days effect $F(4, 52) = 4.62$, $MSE = 4.39$, $p < 0.01$. As it is evident from Figure (10-A), the rats in Sucrose-Locked Wheel group consumed significantly less food than the rats in Sucrose-Wheel group. This might be expected as the rats in these two groups were consuming very different amounts of sucrose (see below).

*Food intake of weanlings:*

The food consumption of weanlings for the first five days was also analyzed using a 2 × 2 × 5 mixed ANOVA, and found a significant main effect of sucrose, $F(1, 28) = 50.98$, $MSE = 12.37$, $p < 0.01$, and days, $F(4, 112) = 65.51$, $MSE = 1.43$, $p < 0.01$. The

only significant interaction was wheel × sucrose, $F(1, 28) = 6.53, p < 0.05$. Figure (10-B) shows the food intake of weanlings. The significant sucrose effect suggests that the food intake of the weanlings with sucrose access was less than that of the weanlings with no sucrose access, perhaps due to the sucrose consumption (see below).

A subsequent 2 × 5 ANOVA for the groups with no sucrose access found only a significant days effect $F(4, 56) = 19.25$, $MSE = 1.86$, $p < 0.01$. The rats in the No Sucrose-Wheel and in the No Sucrose-Locked Wheel groups increased their food intake over the first five days of the study and wheel access had no impact on their feeding.

Similar planned ANOVA for the groups with sucrose access also revealed only a significant days effect $F(4, 56) = 60.87$, $MSE = 1.0$, $p < 0.01$. As it is shown in Figure (10-B), both Sucrose-Wheel and Sucrose-Locked Wheel group rats suppressed their food intake due to consumption of sucrose on first day relative to the other two groups with no sucrose access, however, both groups increased their food intake over subsequent days. It is important to note that wheel access did not influence the food consumption of the weanlings as it did for the adults, and this was over the first five days of the experiment during which weanlings were running as much as adults.

For the last five days, a similar planned ANOVA of the weanlings' feeding found a marginal main effect of wheel, $F(1, 28) = 4.20$, $MSE = 21.59$, $p = 0.05$, significant main effects of sucrose, $F(1, 28) = 38.7$, $p < 0.01$, and days, $F(4, 112) = 64.97$, $MSE = 1.58$, $p < 0.01$. The days × sucrose, $F(4, 112) = 4.12$, $p < 0.01$, and days × wheel interactions, $F(4, 112) = 4.05$, $p < 0.01$ were significant. The wheel × sucrose interaction approached significance, $F(1, 28) = 4.14$, $p = 0.51$. This was followed by two planned ANOVAs looking at groups with and without sucrose access separately.

For the groups with no sucrose access there was only a significant days effect $F(4, 56) = 36.71$, $MSE = 1.98$, $p < 0.01$. As shown in Figure (10-B) both groups of weanlings without sucrose access increased their food intake over the last five days and wheel access was not important.

For the groups with sucrose access, there was a significant effect of wheel, $F(1, 14) = 6.17$, $MSE = 29.17$, $p < 0.05$, days $F(4, 56) = 30.9$, $MSE = 1.18$, $p < 0.01$, and days × wheel interaction, $F(4, 56) = 5.84$, $p < 0.01$. As it is shown in Figure (10-B), weanling rats in both Sucrose-Locked Wheel and Sucrose-Wheel conditions increased their food intake over the last five days. However, the weanlings in Sucrose-Locked Wheel condition consumed less food than the weanlings in Sucrose-Wheel condition. This is surprising both because wheel running did not influence food intake in the first half of the study, and because animals with wheel access were consuming more food.

*Sucrose consumption:*

The sucrose consumption of the 4 groups with sucrose access was analyzed using a 2 (age) × 2 (wheel) × 5 (days) mixed ANOVA. For the first 5 days of the experiment, the ANOVA found a significant main effect of wheel, $F(1, 27) = 34.64$, $p < 0.01$, and days, $F(4, 108) = 10.43$, $MSE = 59.28$, $p < 0.01$. The age × wheel interaction, $F(1, 27) = 11.91$, $p < 0.01$ was also significant. Figure 11 shows that, in the Locked Wheel conditions, weanlings consumed less sucrose than the adult rats, but in the Wheel conditions they consumed more sucrose than the adults. Subsequent planned 2 (wheel) × 5 (days) ANOVAs were performed to look at sucrose consumption of each age group separately.

For the adults, the ANOVA found both a significant main effect of wheel, $F(1, 13) = 32.98$, $MSE = 1181.64$, $p < 0.01$, and days, $F(4, 52) = 5.24$, $MSE = 66.74$, $p < 0.01$. Similar ANOVA for the weanlings found only a significant effect of days, $F(4, 56) = 7.37$, $MSE = 52.36$, $p < 0.01$. This indicates that wheel access suppressed sucrose intake of adults but not the sucrose consumption of the weanlings.

It is important to note that 5 out of 8 weanlings with wheel access consumed approximately the same amount of sucrose as did the weanlings without wheel access. These 5 weanlings with wheel access consumed an average of 34.6 ± 3.7 g (range 19.3 to 43.4) of sucrose, and the weanlings without wheel access consumed an average of 37.1 ± 2.9 (range 29.7 to 52.3) g of sucrose over the first five days. There were only 3 weanlings that showed a marked sucrose suppression and consumed only an average of 9.6 ± 3.8 g (7.6 to 11.0) of sucrose over the first five days.

For the last 5 days of the study, the 2 (age) × 2 (wheel) × 5 (days) mixed ANOVA revealed a significant main effect of wheel, $F(1, 27) = 25.1$, $MSE = 1152$, days, $F(4, 108) = 15.02$, $MSE = 79.49$, $p < 0.01$, and age × wheel interaction, $F(1, 27) = 6.5$, $p < 0.05$. The subsequent planned 2 (wheel) × 5 days ANOVA of the sucrose intake for the adults found a significant main effect of wheel, $F(1, 13) = 22.82$, $MSE = 1394.21$, $p < 0.01$, and days, $F(4, 52) = 5.22$, $MSE = 122.17$, $p < 0.01$. However, similar ANOVA for the weanlings found only a significant effect of days, $F(4, 56) = 17.47$, $MSE = 39.86$, $p < 0.01$. This indicated that for days 6 to 10 wheel running did not effect sucrose consumption of weanlings but it did suppress sucrose consumption for the adults.

Figure 11 shows the mean sucrose intake of the adult and weanling rats with or without wheel access for the entire 10 days of the experiment. As evident from Figure 11

while there is a large difference between sucrose consumption for the two adult groups, the difference between sucrose intake for the two weanling groups is comparatively small. In other words, wheel running only suppressed sucrose intake for adults and not for weanlings. Note that sucrose intake of the weanlings with wheel access is slightly lower than that of the weanlings with locked wheels. Even though the sucrose consumption difference was not significant, it may explain the difference in the food consumption of these groups over the last five days of the study. The weanlings in Sucrose-Wheel condition consumed somewhat less sucrose than the weanlings in Sucrose-Locked Wheel condition, which may have influenced their food consumption (see below, total daily calorie intake of weanlings).

*Total daily energy (calorie) intake of adults:*

Since some rats had two sources of calorie intake (sucrose solution and chow), it was reasonable to investigate total daily energy intake. The total daily calorie intake for the groups with no sucrose access was calculated by multiplying the amount of food consumed by 2.98 (metabolizable energy of rat chow = 2.98 Kcal/g). Total daily calorie intakes of the groups with two sources of energy (rat chow and 32% sucrose solution) were calculated as in previous experiments. These data are shown for adults and weanlings in Figure 12 A and B respectively. (Because there is only one food sources during baseline these results are the same as food consumption data presented previously).

The total energy intake data in adults were analyzed with a 2 × 2 × 5 mixed ANOVA for the first five days and revealed a significant main effect of wheel, $F(1, 27) = 63.62$, $MSE = 751.35$, $p < 0.01$, sucrose, $F(1, 27) = 35.83$, $p < 0.01$, and days, $F(4, 108) =$

28.38, $MSE = 111.46$, $p < 0.01$. The sucrose × wheel, $F(1, 27) = 9.6$, $p < 0.01$, and days × wheel, $F(4, 108) = 6.12$, $p < 0.01$ interactions were significant. To explore these results further, groups of animals with and without sucrose availability were analyzed separately. As rats with no sucrose had only food their total calorie consumption is the same as the consumption of food and thus for these groups the reader is referred back to the food data.

A planned ANOVA was performed to look at groups with sucrose and food access for the first five days, and revealed a significant wheel effect $F(1, 13) = 31.54$, $MSE = 1412.31$, $p < 0.01$, and days effect $F(4, 52) = 17.94$, $MSE = 114.86$, $p < 0.01$. As it is evident from Figure (12-A), there was a significant difference in total calorie intake of the two groups. The total calorie intake of the rats in Sucrose-Locked Wheel condition increased on first day after baseline and stayed high. The Sucrose-Wheel group showed a temporary suppression of their total calorie intake with recovery by the end of day 5. However, the lack of a significant days × wheel interaction indicates that the total calorie intake of the Sucrose-Wheel group did not increase to the total calorie intake of the Sucrose-Locked Wheel group. This could be expected, because unlike the rats in Sucrose-Locked Wheel group, the rats in Sucrose-Wheel group were not consuming their sucrose solution (see above - sucrose intake).

A 2 × 2 × 5 mixed ANOVA for the total calorie intake was performed for the last five days of the study, and found a significant main effect of wheel, $F(1, 27) = 16.05$, $MSE = 930.62$, $p < 0.01$, sucrose, $F(1, 27) = 35.88$, $p < 0.01$, and days, $F(4, 108) = 7.41$, $MSE = 129.93$, $p < 0.01$. The sucrose × wheel, $F(1, 27) = 7.2$, $p < 0.05$, and days × wheel, $F(4, 108) = 2.57$, $p < 0.05$ interactions were all significant. A planned ANOVA

for those groups with sucrose and food access revealed a significant wheel effect $F$ (1, 13) = 13.58, $MSE$ = 1481.09, $p < 0.01$, and days effect $F$ (4, 52) = 3.86, $MSE$ = 198.04, $p < 0.01$. As it is shown in Figure (12-A), there was a significant difference in total calorie intake of the two groups with sucrose access. Both groups showed a changing total calorie intake over the last five days of the study, however, total calorie intake of the rats in the Sucrose-Locked Wheel condition was considerably higher than that of the rats in the Sucrose-Wheel condition, since the rats with wheel access were consuming mostly their familiar rat chow, and hardly any of the novel sucrose solution.

*Total daily energy (calorie) intake of weanlings:*

Similar ANOVA for the first five days of weanling calorie intake revealed a significant main effect of sucrose, $F(1, 28) = 77.54$, $MSE = 406.3$, $p < 0.01$, and days, $F(4, 112) = 34.49$, $MSE = 47.09$, $p < 0.01$. Only days × sucrose, $F(4, 112) = 6.53$, $p < 0.01$ interaction was found to be significant. A planned ANOVA for the groups with sucrose and food access revealed only a significant days effect $F$ (4, 56) = 20.78, $MSE$ = 77.7, $p < 0.01$. As it is shown in Figure (12-B), both groups increased their total calorie intake over the first five days as they became older, and wheel access did not influence their total calorie intake (The total daily energy intake of the No Sucrose-Locked Wheel and No Sucrose-Wheel group rats is the same as their food consumption data presented previously).

For the last five days of the study, a 2 × 2 × 5 mixed ANOVA for the total calorie intake was performed, and found a significant main effect of sucrose, $F(1, 28) = 90.95$, $MSE = 687.53$, $p < 0.01$, and days, $F(4, 112) = 9.28$, $MSE = 41.55$, $p < 0.01$. The days × sucrose, $F(4, 112) = 9.28$, $p < 0.05$ interaction was also significant. A planned ANOVA

for the groups with sucrose access found only a significant days effect $F$ (4, 56) = 39.71, $MSE$ = 65.5, $p < 0.01$. As it is shown in Figure (12-B), both groups of rats with sucrose access were increasing their total calorie intake over the last five days of the study similarly and regardless of wheel availability. (The total daily energy intake of the No Sucrose-Locked Wheel and No Sucrose-Wheel group rats is the same as their food consumption data presented previously).

*Last day body weight:*

A 2 × 2 ANOVA to look at weight separately for the weanling and adult rats over the last day of the study (day 10) was performed. No significant difference was found among the body weights of the four groups of weanlings on day 10. On the last day of the study, the Sucrose-Locked Wheel group weanlings weighed 164.81 ± 17.5 g, the Sucrose-Wheel group weanlings weighed 172.75 ±8.9 g, the No Sucrose-Locked Wheel group weighed 170.38 ± 12.2 g, and the No Sucrose-Wheel group weanlings weighed 168.19 ± 8 g.

For adults, there was only a significant effect of wheel, $F(1, 27) = 9.5$, $MSE$ = 430.78, $p < 0.01$ on body weight. Both groups of adults with wheel access weighed considerably less than the two groups of adults without wheel access, on the last day of the study. The Sucrose-Wheel group weighed 323.71 ± 14.2 g, and the No Sucrose-Wheel group weighed 317.13 ± 12.9g. The Sucrose-Locked Wheel group weighed 347.13 ± 21.3 and the No Sucrose-Wheel group weighed 339.75 ± 29.4 g.

## Discussion

The wheel running results of Experiment 3 were surprising in that there was a significant difference in the development of wheel running between adult and weanling

rats. Even though in the first half of the study, both adults and weanlings showed similar levels of running, in the second half of the study, only the adults increased their running level. This is not consistent with previous studies (Afonso, 2000; Cortright, et al., 1997; Pitts, 1984), in which weanlings have been observed to increase their running level over days from wheel introduction. Although weanlings in this study did not show an increase in running, this study lasted for only 10 days, and an increase in running might have become evident had the study continued further. Another major difference between this study and the Afonso (2000) study was the equipment used. Afonso (2000) used custom-built wheels with no bedding in the cages. In this study, commercially-built wheels were used and there was wood chip bedding in the cages, which sometimes mounts up under the wheels, making them harder to turn for weanlings. However, the focus of this study was the initial effect of running on consumption of both familiar and novel food and no difference in the running level of adults and weanling was evident in the first 5 days. Therefore, any differences in feeding for this period are unlikely to be due to running differences.

Wheel running induced a feeding suppression in adults but it had no effect on either familiar or novel food consumption or total calorie intake in weanlings. The adults with wheel access (regardless of sucrose availability) suppressed their food consumption initially and then increased their consumption to that of no wheel-no sucrose control rats. These results are consistent with previous work (Satvat & Eikelboom, 2003). Wheel running, however, did not influence the food intake of the weanlings. The weanlings with and without wheel access consumed the same amount of food. This is also consistent with previous studies (Afonso 2000; Pitts, 1984). In fact, in the last five days of the study,

the weanling with both wheel and sucrose access consumed more food than the weanlings, with only sucrose access, but total calorie intake of these two groups was found to be similar.

Whereas the adult rats with access to both sucrose and a wheel, completely suppressed their sucrose intake, the weanlings with wheel access did not show any sucrose suppression compared to the weanlings with no wheel access. To elucidate whether the difference between sucrose consumption of the groups with and without wheel access disappears completely in younger rats, it would be of interest to study the effect of wheel running on consumption of a novel food in younger (20 to 25 day old) weanlings.

In summary, the results suggest that whereas wheel running had no effect on consumption of both familiar and novel food in weanlings, it had a suppressive effect on consumption of familiar food and a much more pronounced and stronger suppressive effect on consumption of a novel food in adult rats. The difference between weanling and adults in the effect of wheel running may be due to the rapid growth that weanlings are experiencing, or it may be due to the inability of younger rats to associate the novel food to the unconditioned effect of running.

## General Discussion

The first aim of this thesis, using alternate-day wheel access procedure, was to explore whether a conditioned taste avoidance, an anorectic effect, or both of these effects were responsible for feeding suppression brought about by running. Based on the conditioned taste avoidance hypothesis, wheel running may induce "sickness" and as a result, the animal avoids tastes that have been paired with wheel access. This running-

induced "sickness" has been suggested to be a possible cause of feeding suppression induced by running (Lett & Grant, 1996). On the other hand, running may induce suppression of appetite by a direct anorectic effect (Mueller et al, 1997).

In Experiment 1, the rats with continuous wheel access suppressed their consumption of familiar rat chow relative to baseline days' consumption. They also reduced their novel sucrose consumption relative to animals without wheel access. However, whereas the suppression of the familiar rat chow recovered after only a few days, the intake suppression of the novel sucrose was very strong, almost complete, and long lasting. Similar results, comparing novel and familiar food suppression have been observed previously (Satvat & Eikelboom, 2003).

As in Mueller et al.'s (1997) study of the effect of alternate-day wheel access on food intake, rats in Experiment 1 showed a zigzag pattern of consumption for their familiar rat chow. The animals suppressed their familiar food intake only on the days they had access to the wheel, while on the intervening days with no wheel access, they consumed normal amounts of food. The zigzag pattern of consumption however, was not evident for the intake of the novel sucrose solution. The alternate-day wheel rats showed very low levels of consumption of the sucrose solution regardless of wheel availability. In other words, the rats avoided the sucrose whether they were in the wheels or home cages. Nonetheless, toward the end of the study, these same rats started to consume more sucrose solution on home cage days but maintained their suppressed sucrose intake on wheel days, resulting in a zigzag pattern of sucrose consumption over the last 6 days of the study.

It has been suggested that the initial feeding suppression induced by running might be due to a conditioned taste avoidance (Hayashi et al. 2002; Lett & Grant, 1996; Lett et al., 1998; Lett et al., 2001; Nakajima et al., 2000). The results of Experiment 1 are in agreement with the conditioned taste avoidance hypothesis. Evidently, if the food is novel it supports a more pronounced suppression than when the food is familiar, as would be predicted based on a conditioning explanation. The animals associate the state change, induced by running, with the novel food, rather than the familiar food which is associated with normal affect.

The fact that the group of rats with alternate-day wheel exposure suppressed their intake of the novel sucrose, regardless of wheel accessibility, also supports the conditioned taste avoidance hypothesis. On wheel days, an association is apparently formed between the novel sucrose solution and the unconditioned effect of running. Thus, animals avoid consuming sucrose solution on wheel-days in response to the unconditioned effect of running ("sickness"). The days when rats are back in their home cages, sucrose solution is still avoided but now in response to the learned association between the taste and the unconditioned effects of running. Such an association is not formed when the food is familiar due to latent inhibition (see below). Therefore, the rats consume normal amount of the familiar food when the wheel is not available. However, on wheel days, the unconditioned effect of running occurs and directly influences their food consumption.

CS pre-exposure has been shown to reduce conditioned taste avoidance, a phenomenon called latent inhibition (Lubow & Moore, 1959; Lubow, 1989). Thus the aim of Experiment 2 was to investigate the effect of sucrose pre-exposure on the intake

suppression induced by wheel running. In Experiment 2 only the direct effects of wheel running on consumption (only on wheel days) was expected. Because of sucrose pre-exposure no conditioning was expected to occur. Thus the zigzag pattern of consumption should be evident from the first alternate-day wheel exposure. Also only a much milder suppression of the familiar sucrose solution in the group of rats with continuous wheel access, was expected compared to the strong and long lasting suppression of the novel sucrose solution, seen in Experiment 1.

In terms of consumption, as predicted, sucrose pre-exposure reduced the suppression of sucrose solution considerably in the rats given continuous wheel access. Consumption of both familiar sucrose and rat chow was temporarily suppressed, and recovered after only a few days. The rats with alternate-day wheel exposure showed a zigzag consumption pattern of the now-familiar sucrose throughout the entire study; suppressed on wheel days compared to their more normal consumption on non-wheel days. As the rats were familiarized with sucrose prior to the introduction of the alternate-day wheel access, they did not form association between the unconditioned effect of wheel running and the sucrose solution. Therefore, on non-wheel days rats consumed normal amount of familiar sucrose solution, but on wheel days, the unconditioned effect of running still directly suppressed the consumption of familiar sucrose. For the same reason, these animals showed a zigzag pattern of familiar rat chow intake at the beginning of the experiment.

One study closely related to the present study is that of Puente, Cannon, Best and Carrell (1988), where a familiar fluid (tap water) and sickness, induced by LiCl injection, were paired in a distinctive context. On intervening home cage days, rats were not

injected with LiCl, but still had access to the same tap water. Over trials, this resulted in reduction of fluid intake in the context that was paired with LiCl but not in the home cages. In a second experiment, Puente et al. (1988) found that consumption of a novel fluid that had not been paired with LiCl was not suppressed in the LiCl context. It was suggested that the expression of a taste avoidance can be controlled by environmental cues. The same rationale may apply to the zigzag pattern seen in both Experiments 1 and 2 for consumption of familiar foods. During wheel days, contextual cues that were paired with unconditioned effects of running signaled avoidance of familiar food and sucrose solution, and during non-wheel, home cage days, the familiar context, which was not paired with unconditioned effects of wheel running, signaled familiar food and sucrose solution as a safe source of food. In other words, contextual cues paired with unconditioned effects of wheel running may have controlled all familiar food avoidance.

Taken together, the results of Experiments 1 and 2 indicate that wheel running supports a conditioned taste avoidance, as has been previously suggested (Lett & Grant, 1996). The consumption of a novel food paired with wheel access is completely suppressed, whether the rats have continuous or alternate-day wheel access. The consumption of familiar food is suppressed only on wheel days and for a shorter period of time when rats are introduced to the wheel.

However, even though, the results show that wheel running can act as an unconditioned stimulus to support the development of CS-US association, this does not directly indicate what internal changes are induced by wheel running. As has been mentioned, Lett and Grant (1996) suggested that wheel running may produce "sickness". It is also possible that wheel running may induce a euphoria-like state similar to that

brought about by reinforcing drugs (Eikelboom & Lattanzio, 2003). Rewarding drugs such as cocaine or amphetamine, which are self-administered and support a conditioned place preference, have also been shown to support a conditioned taste avoidance when paired with an otherwise palatable novel taste (Hunt & Amit, 1987).

A number of lines of evidence suggest that wheel running may induce a state similar to addictive drugs. Rats lever press to gain wheel access (Iverson, 1993). Similarly, rats will lever press to receive an infusion of cocaine. With ad lib access in cocaine and heroin self-administration, the rate of self-administration increases over days, and if ad lib access continues, rats die from an overdose (Bozarth & Wise, 1985). This escalation in consumption over days is a classic feature of addictive behaviour, and parallels the transition to addiction in humans. Similarly, with ad lib wheel access; rats come to increase their running over a number of days to an excessive level (Eikelboom & Mills, 1988).

Rats develop a conditioned place preference for an environment associated with the after-effects of both wheel running (Lett, Grant, Byrne, & Koh, 2000) and addictive drugs such as cocaine (Mucha, Bucenieks, O'Shaughnessy, & van der Kooy, 1982). Immediately after wheel access, Lett and colleagues (2000) placed rats on one distinctive side of a chamber divided by a barrier. On days without wheel access, rats were placed on the other side. Later, at test, the barrier was removed and the rats had free access to both sides. The side that was paired with wheel running was preferred. This suggested that wheel running produced a positive affect that outlasted the wheel access and supported a place preference.

Administration of naloxone, a mu-opioid receptor antagonist, attenuates the conditioned place preference induced by both wheel running and opiates, suggesting that the rewarding properties of both wheel running (Lett, Grant, & Koh, 2001) and morphine are mediated by the endogenous opioid system (Akil, Watson, Young, Lewis, Khachaturian, & Walker, 1984). Abstaining from addictive drugs produces withdrawal symptoms (Kantak & Miczek, 1986). Similarly, there is evidence that locking the wheels after chronic wheel access produces withdrawal symptoms such as aggression in rats (Hoffmann, Thoren, & Ely, 1987). Wheel running seems to have parallels with many aspects of drug self-administration. This has led to the suggestion that wheel running may serve as an animal model of addiction (Eikelboom & Lattanzio, 2003; Lattanzio & Eikelboom, 2003).

From the present experiments, it is not possible to determine whether the novel food avoidance induced by wheel running is similar to the novel food avoidance induced by reinforcing drugs, such as amphetamine, or if it is similar to that induced by illness inducing drugs, such as LiCl. Using the taste reactivity test, it has been demonstrated that the taste avoidance produced by illness-inducing agents is qualitatively different from the taste avoidance induced by reinforcing drugs (Parker, 1995). The taste reactivity test measures the behavioural response of the animals to a flavour that is delivered through an intraoral cannula into the mouth. "Gaping, paw treading, and chin rubbing" in rats are the distinctive disgust reactions to a novel flavour, which is intrinsically aversive (quinine) or which has been paired with illness-inducing agents. It was shown with the taste reactivity test that these disgust reactions are elicited only when the flavoured solution has been paired with emetic drugs. When the flavoured solution is paired with reinforcing drugs,

and then delivered to the mouth by cannula, these disgust reactions are not evoked. The flavoured solution elicits ingestive reactions including "tongue protrusions, mouth movement and paw lick". Therefore, whereas the reinforcing drugs produce only conditioned taste avoidance, the illness-inducing agents produce conditioned taste avoidance with aversion in a taste reactivity test (Parker, 1995).

The taste reactivity test can thus demonstrate if avoiding the novel solution, paired with wheel running, is the result of illness or not. If "sickness" is responsible for avoiding sucrose consumption, then reactions to the taste, infused to the mouth through cannula, should be similar to those of seen with illness-inducing agents (disgust reactions). However, if the animals react positively to the solution infused to their mouth, then the avoidance induced by running resembles the avoidance induced by rewarding drugs. Certainly, such a study may elucidate whether the unconditioned effect of wheel running is "sickness" or a positive affect.

The second aim of this thesis was to explore the effect of age on the wheel running-induced suppression of novel food. Previous studies have shown that despite the large weight difference between the adults and weanlings, acquisition of wheel running in adult and weanling rats was similar (Afonso, 2000). Both weanlings and adults increased their running at the same rate and reached a running plateau at the same level. Experiment 3 revealed slightly different results with respect to wheel running acquisition in weanlings and adult rats. In the first few days, both adults and weanlings were running similar amounts, but in the last five days, only the adults increased their running. This was not consistent with previous studies (Afonso, 2000; Cortright et al., 1997; Pitts, 1984), in which weanlings were observed to increase their running over days and reached

a plateau after about 50 days of wheel access. The weanlings in the present study ran a stable amount over the whole study. It is not clear whether the weanlings would have increased their running, had the study continued longer. The difference found in the pattern of running in the present study and that of Afonso's (2000) may also be due to different equipment used in these studies (custom wheels vs. commercial Nalgene wheels). However, as the focus of Experiment 3 was on the initial effect of running on consumption and no difference in the running level of adults and weanling was evident over the first 5 days, the subsequent difference in running is unlikely to be involved in the early feeding differences.

Experiment 3 supports the finding that wheel running induced a feeding suppression only in adults and did not influence consumption of familiar food in weanlings (Afonso, 2000). With the novel sucrose, whereas the adult rats with wheel access completely suppressed their sucrose intake compared to the rats with no wheel access, the weanlings with wheel access showed only a trivial, non-significant, suppression of sucrose consumption. The difference between sucrose consumption of the two groups of adult rats with and without wheel access was considerably larger. The weanlings in the present study were about 28 to 31 days of age at the start of the experiment. Because three out of eight weanlings with wheel access showed a sucrose suppression, it would be of interest to study the effect of wheel running on consumption of a novel food in younger rats. This would show whether the difference between sucrose consumption of the groups with and without wheel access would disappear completely in younger rats, and whether the wheel running induced- suppression of novel food appears gradually over time and becomes more evident during adulthood.

Afonso (2000) suggested that because weanlings are in a rapid growth period, wheel running may not affect their food consumption. It was suggested that hormones such as growth hormones may be active during this early developmental stage and block the mechanism responsible for food suppression. However, if wheel running truly induces "sickness" as a side effect in adult rats, then weanlings may also experience running-induced sickness, and another explanation may be needed.

Infant rats have shown to be capable of associative learning (Campbell & Alberts, 1979; Gemberling, Domjan, & Amsel, 1980), but the results of Experiment 3 revealed that weanlings did not suppress their consumption of a novel sucrose solution that was paired with wheel running. It is important to note that emetic drug (LiCl) has been used as an US in the studies of conditioned taste aversion learning in infant rats (Campbell & Alberts, 1979; Gemberling et al., 1980). It is possible that the nausea induced by LiCl may be more severe than the state change induced by running. Nevertheless, the adults ran as much as the weanlings in the first half of the study, but they completely suppressed their sucrose intake, suggesting that the unconditioned effect of wheel running (whether "sickness" or a more positive hedonic effect) should have been effective enough to induce a similar effect, if not more, in the weanlings.

Another possible explanation for the lack of a feeding suppression (both with familiar and novel food) in weanlings is a lack of CS-US contingency. In the present study animals had ad lib access to food, sucrose and wheel. One important factor in the development of a conditioned taste avoidance is CS-US interval, and weanlings have shown to be especially sensitive to the CS-US intervals (Gemberling, et al., 1980; Gregg, Kittrell, Domjan, & Amsel, 1978). Five to twelve day old rats were found to be unable to

learn association with a CS-US interval longer than 30 minutes. Fifteen day old rats however, could learn association with CS-US intervals as great as 2 hours (Gemberling et al., 1980; Gregg et al., 1978). Long-delay conditioned taste avoidance in adult rats has shown to be superior to that of weanlings (20 to 24 days) (Misanin, Grieder, & Hinderliter, 1988). Old adult rats (18 to 27 months) are even superior to young adult rats (2 to 5 months) in learning CS-US association over longer CS-US intervals (Hinderliter & Misanin, 1995).

The weanlings in Experiment 3 were 28 to 31 days of age and the adults were approximately 2 to 3 months old. The above studies (Campbell & Alberts, 1979; Gemberling et al., 1980; Gregg et al., 1978; Misanin et al., 1988) suggest that the formation of a conditioned taste avoidance over long delays has a developmental aspect. Thus, presumably the weanlings in Experiment 3 were inferior to adult rats in learning CS-US association. In Experiment 3 rats were given ad lib food, sucrose and wheel, but their pattern of consumption was not recorded. Therefore, if the weanling rats did not consume sucrose immediately prior to running, formation of the conditioned taste avoidance may have been weak. It is interesting to note that five out of eight weanlings with wheel access in Experiment 3 were consuming more or less the same amount of sucrose as the weanlings without wheel access. There were only three weanlings that showed a marked sucrose suppression. It is possible that initially these three rats happened to consume sucrose solution immediately prior to running. But the other five weanlings, which did not show any sucrose suppression, might have had experienced a slightly different schedule: they might have run first and then consumed the novel sucrose (backward conditioning), or the time interval between the sucrose consumption

and running may have been too long (delayed CS-US interval). Further studies, using classical procedure of taste conditioning, similar to studies done with adults (Lett & Grant, 1996) clearly are needed to elucidate the effect of wheel running on consumption of both novel and familiar food in weanlings.

In addition, whereas in weanlings, wheel running did not induce intake suppression, sucrose consumption induced a suppression of rat chow intake in the weanlings regardless of wheel access. The rats of both ages preferred sucrose to rat chow and thus eat less chow. However, sucrose consumption induced a suppression of rat chow intake only in the adult rats with no wheel access. Sucrose consumption did not influence rat chow intake of the adults with wheel access, since these rats completely avoided their sucrose.

As mentioned earlier, there is a body of evidence that suggests wheel running may induce a state similar to addictive drugs (Eikelboom & Lattanzio, 2003). Moreover, paradoxically, rewarding drugs, when paired with a novel flavoured solution, have shown to induce a conditioned taste avoidance in adult rats (Hunt & Amit, 1987). The effect of addictive drugs in producing conditioned taste avoidance in weanlings has not been well investigated. It is not clear whether or not drugs such as morphine or amphetamine, when paired with an otherwise novel palatable flavour, induce a conditioned taste avoidance in weanling rats as they do in adult rats. Such studies need to be done with weanlings to investigate parallels between addictive drugs and wheel running in infant rats.

When a rat is given ad lib wheel access simultaneously with one hour food access a day, running is facilitated, and the feeding suppression results in the rats literally starving themselves to death (Epling & Pierce, 1992; Finger, 1951; Routtenberg &

Kuznesof, 1967). This combination of ad lib wheel access and restricted food access is referred to as "activity-based anorexia" and has been suggested as an animal model of anorexia nervosa (Epling & Pierce, 1992). It seems that the marked decrease in consumption and decline in caloric intake results in progressively more running, which further reduces food intake, in a vicious cycle (Epling & Pierce, 1992). No other activities combined with restricted food are known to produce anorexia in rats. For example, Koh, Lett, & Grant (2000) presented rats with a circular alley and restricted food, but found no evidence of anorexia in these animals. However, the potential of alternate behaviours in the production of anorexia have not been well investigated.

Development of activity-based anorexia in weanlings has not been extensively explored. Paré (1975) compared the development of activity-stress ulcers in three different age groups (but described animals only by weights; 100, 200, and 300 g). The procedure of activity-stress is similar to that of activity anorexia and includes ad lib wheel in combination of only one hour daily food access (the difference is in what the focus of these studies is: weight or stomach ulcers) . Paré (1975) found that younger rats, which ran more, died sooner than the older rats, which ran less. In this study young animals did not reduce their food intake during the one hour daily food access and consume a similar amounts of food as control sedentary rats. The author suggested that high level of running rather than reduced food consumption may have been responsible for fatality in these rats. In humans, anorexia nervosa rarely develops before puberty, but it is not clear whether or not children are protected from this disorder. Further research is clearly needed to understand why weanlings do not show the wheel-induced feeding suppression, and what is responsible for this developmental change.

The incidence of anorexia nervosa in today's society seems to be increasing. There is a growing interest in exercise for physical fitness, for prevention and treatment of different disorders, and for general health. Moreover, thinness is highly valued, and dieting seems to be very common in some groups. The combination of dieting and exercise may lead to anorexia nervosa in human. People diagnosed with anorexia not only exercise excessively but also reduce their food consumption markedly (Eisler & le Grange, 1990; Epling & Pierce, 1992). Thus, understanding the underlying physiological mechanisms involved in feeding suppression induced by wheel running may contribute to understanding and treating anorexia nervosa.

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## Figure Captions

*Figure 1.* Mean (±SEM) daily number of wheel turns of rats in Experiment 1: Both Wheel and Alternate-W group rats ran similarly and increased their running level at the same rate.

*Figure 2.* Mean (±SEM) grams of food consumed in Experiment 1: Food intake of the Home group rats was suppressed on day 1. The Wheel group showed an initial feeding suppression and then recovered. The Alternate-W group rats showed a zigzag pattern of food intake, suppressing their food on wheel days and showing normal consumption on non-wheel days.

*Figure 3.* Mean (±SEM) grams of sucrose solution consumed in Experiment 1: The Home group rats consumed a large amount of sucrose solution from the first day. Both the Wheel and Alternate-W group rats suppressed sucrose consumption at the start of the study. By the end of the experiment, the Wheel group showed some recovery of sucrose intake. The Alternate-W showed a zigzag pattern of sucrose consumption, with intake suppression on wheel days, and increased consumption on non-wheel, home cage days.

*Figure 4.* Mean (±SEM) total calories consumed in Experiment 1: Total calorie intake of the Home group rats was elevated on first day of the experiment and stayed high. The Wheel group showed an initial calorie intake suppression, which recovered by the end of the study. The Alternate-W group rats showed a zigzag pattern of calorie intake,

suppression on wheel days and recovery on non-wheel, home cage days throughout the study.

*Figure 5.* Mean (±SEM) daily number of wheel turns of rats in Experiment 2: Both Wheel and Alternate-W group rats ran similarly and increased their running level at the same rate.

*Figure 6.* Mean (±SEM) grams of food consumed in Experiment 2: The rats did not differ in their food consumption during sucrose pre-exposure. Food intake of the Home group rats gradually declined as sucrose consumption increased. The Wheel group showed an initial feeding suppression and then a recovery. The Alternate-W group rats showed a zigzag pattern of food intake, suppression of food on wheel days and recovery on non-wheel days during the first few blocks. By the end of the study the three groups of rats were consuming more or less similar amount of food.

*Figure 7.* Mean (±SEM) grams of sucrose solution consumed in Experiment 2: The three groups of rats consumed similar amount of sucrose solution during baseline. When the wheel was introduced, the Wheel group showed only an initial sucrose suppression with a rapid recovery. The Alternate-W group showed a zigzag pattern of sucrose consumption throughout the study.

*Figure 8.* Mean (±SEM) total calories consumed in Experiment 2: Total calorie intake of the Home group rat did not change over the study. The Wheel group showed an initial

calorie intake suppression at wheel introduction, which was completely recovered by the end of the study. The Alternate-W group rats showed a zigzag pattern of consumption, suppression on wheel days and recovery on non-wheel, home cage days throughout the experiment.

*Figure 9.* Mean (±SEM) daily number of wheel turns of adult and weanling rats in Experiment 3: All four groups of rats ran similarly during the first half of the experiment. During the second half of the study, both groups of adult rats increased their running. Both groups of weanlings did not change their running level over the experiment.

*Figure 10.* A. Mean (±SEM) grams of food consumed by adult rats in Experiment 3: The No Sucrose-Locked Wheel group did not change their food intake over the study. Food intake of the rats in the Sucrose-Locked Wheel group was suppressed at sucrose introduction and stayed low throughout the study. Wheel introduction induced a similar initial feeding suppression and recovery in both groups of rats with wheel access. B. Mean (±SEM) grams of food consumed by weanlings in Experiment 3: Both groups of weanlings without sucrose access consumed and increased their food intake in the same way. The two groups of weanlings with sucrose access suppressed their feeding after sucrose introduction. Wheel running did not, but sucrose access did, influence the food consumption of the weanling rats.

*Figure 11.* Mean (±SEM) grams of sucrose consumed by adult and weanling rats in Experiment 3: There was a significant difference between sucrose intake of the adult rats

with and without wheel access. Adult rats without wheel access consumed a high amount of sucrose, but adults with wheel access showed a strong and long lasting sucrose suppression. Weanlings with and without wheel access differed in their sucrose consumption only marginally.

*Figure 12.* A. Mean (±SEM) total calories consumed by adults in Experiment 3: Total calorie intake of the adults in the No Sucrose-Locked Wheel group did not change from baseline. At sucrose introduction, calorie intake of the rats in the Sucrose-Locked Wheel group increased and stayed high. Both groups of adults with wheel access showed similar initial calorie intake suppression with a slow recovery. B. Mean (±SEM) total calories consumed by weanlings in Experiment 3: Total calorie intake of both groups of weanlings with no sucrose access increased similarly as the rats grew. Total calorie intake of both groups of weanlings with sucrose access was much higher than the other two groups. Wheel access did not influence the calorie intake of weanlings.

**EXPERIMENT 1: FIGURES**

**FIGURE 1 – Wheel Turns**

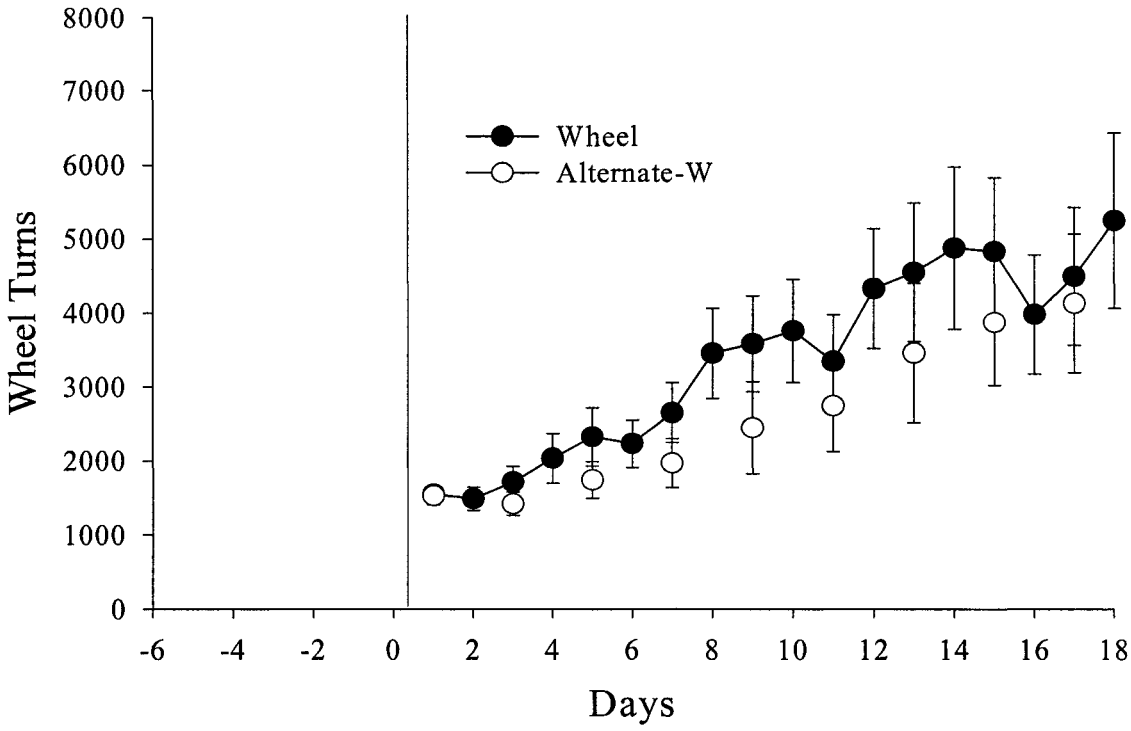


**FIGURE 2 – Food Intake**

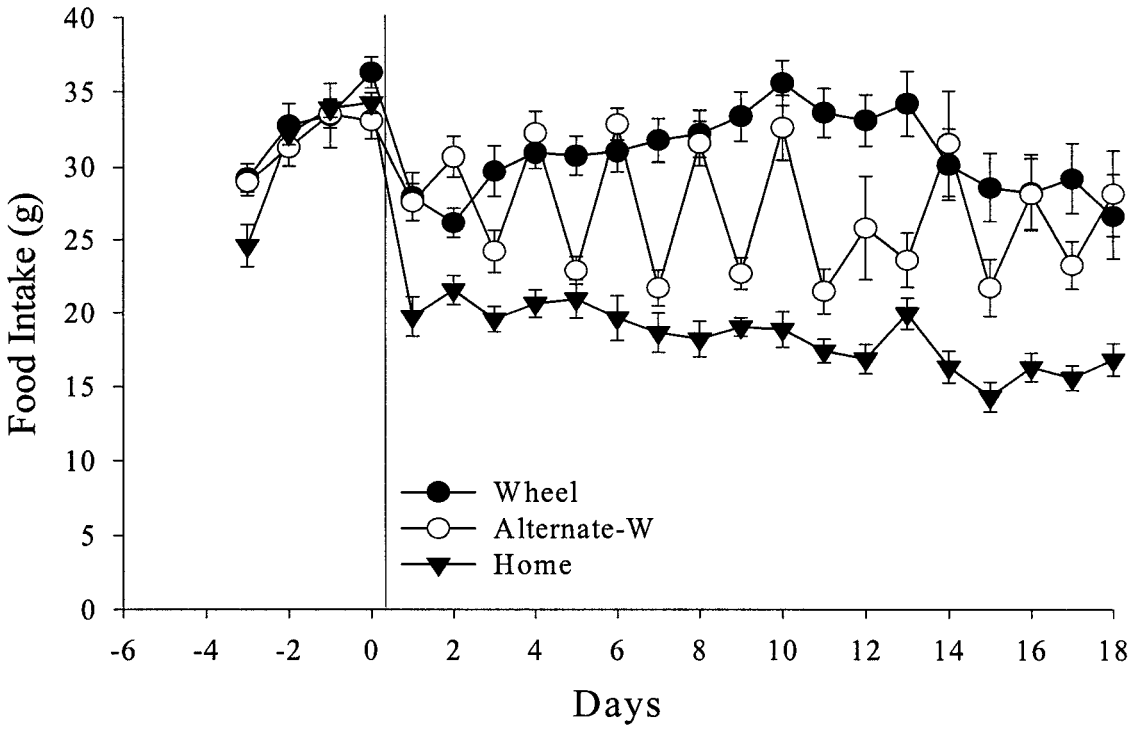


**FIGURE 3 – Sucrose Intake**

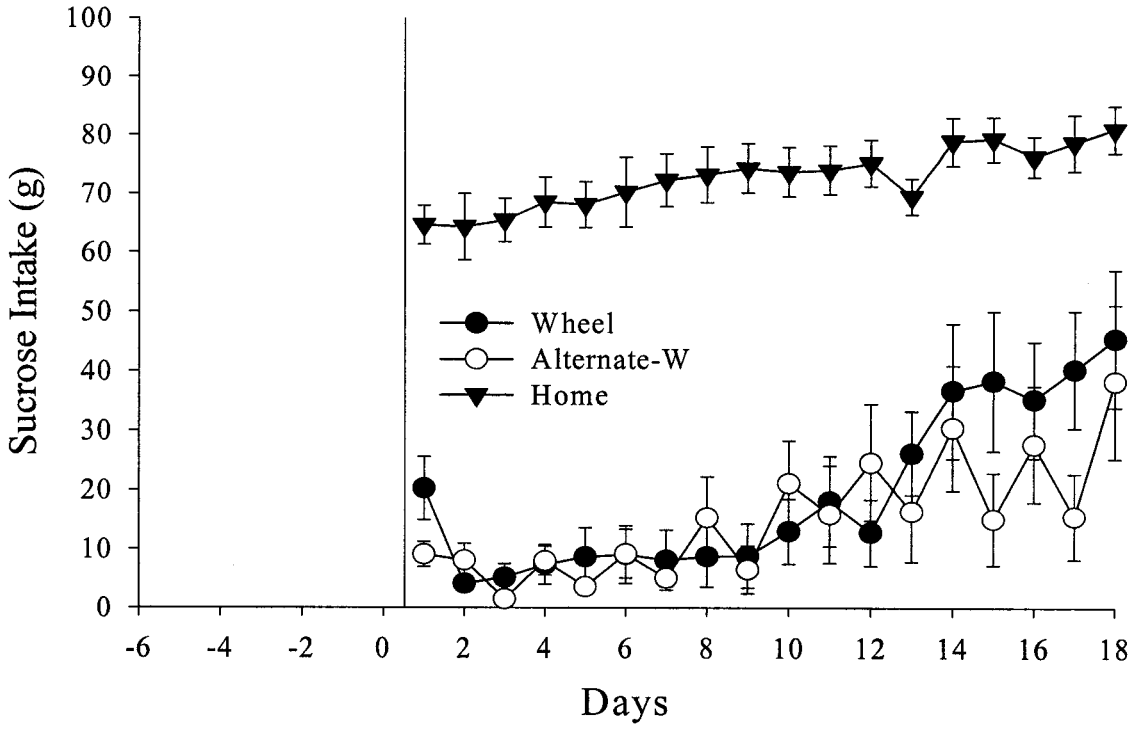
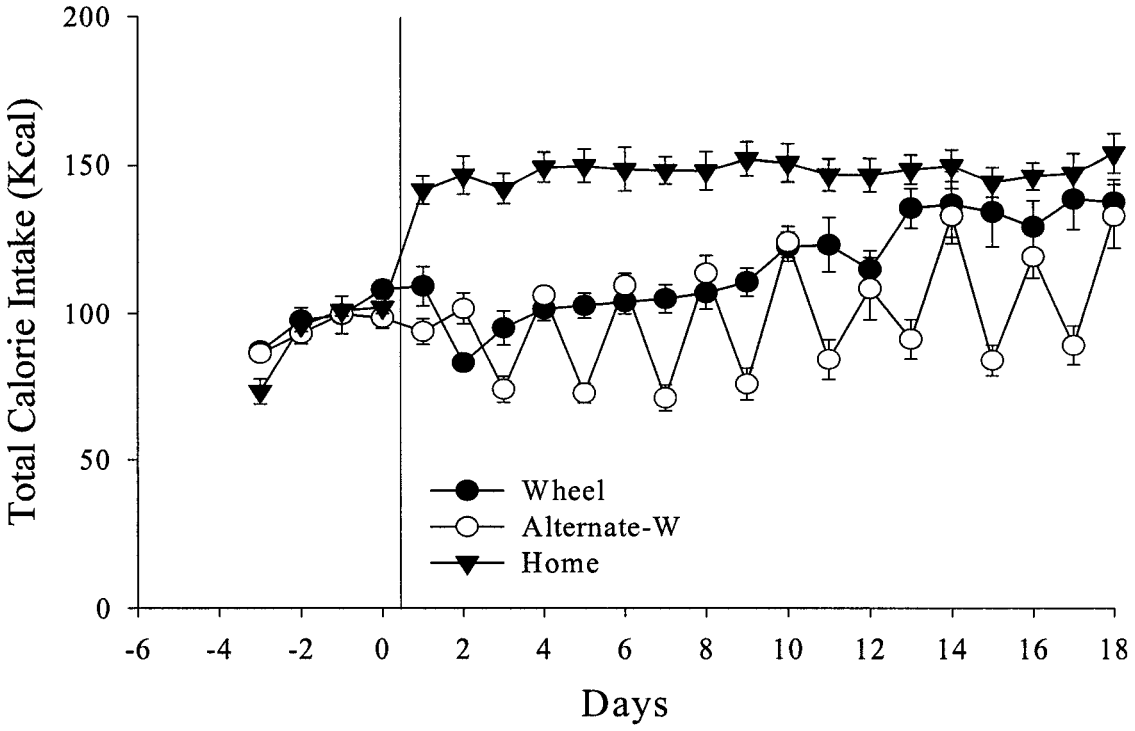


**FIGURE 4 – Total Calorie Intake**



## EXPERIMENT 2: FIGURES

### FIGURE 5 – Wheel Turns

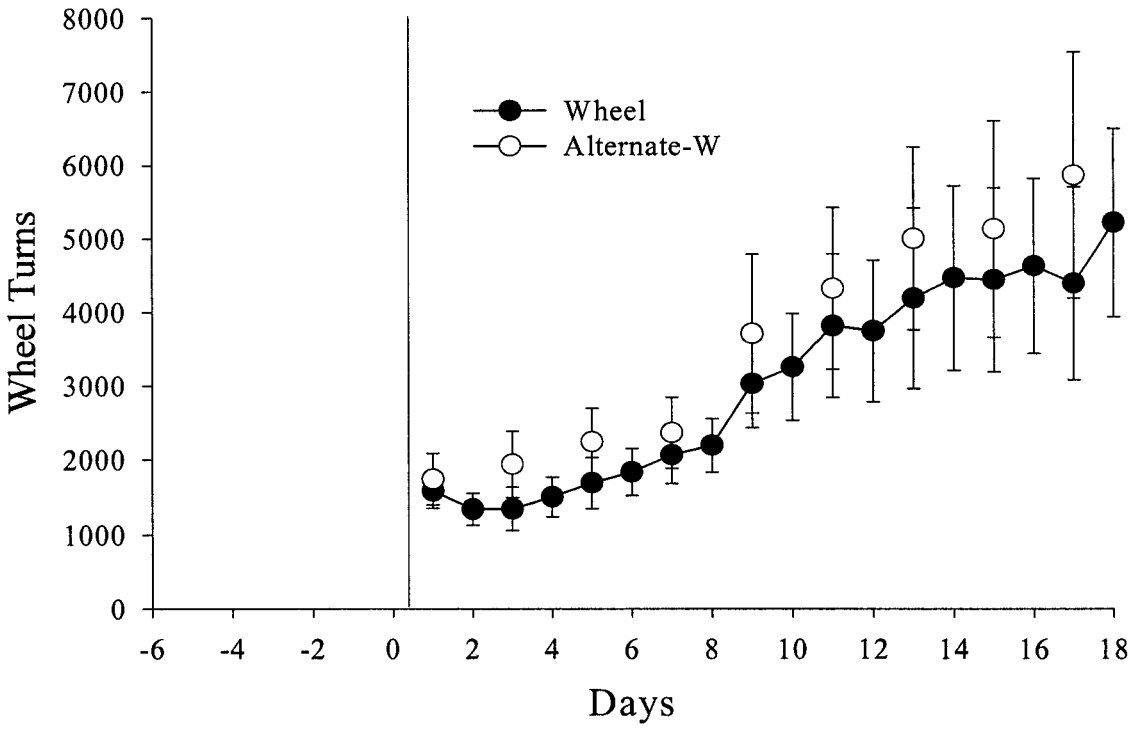


**FIGURE 6 – Food Intake**

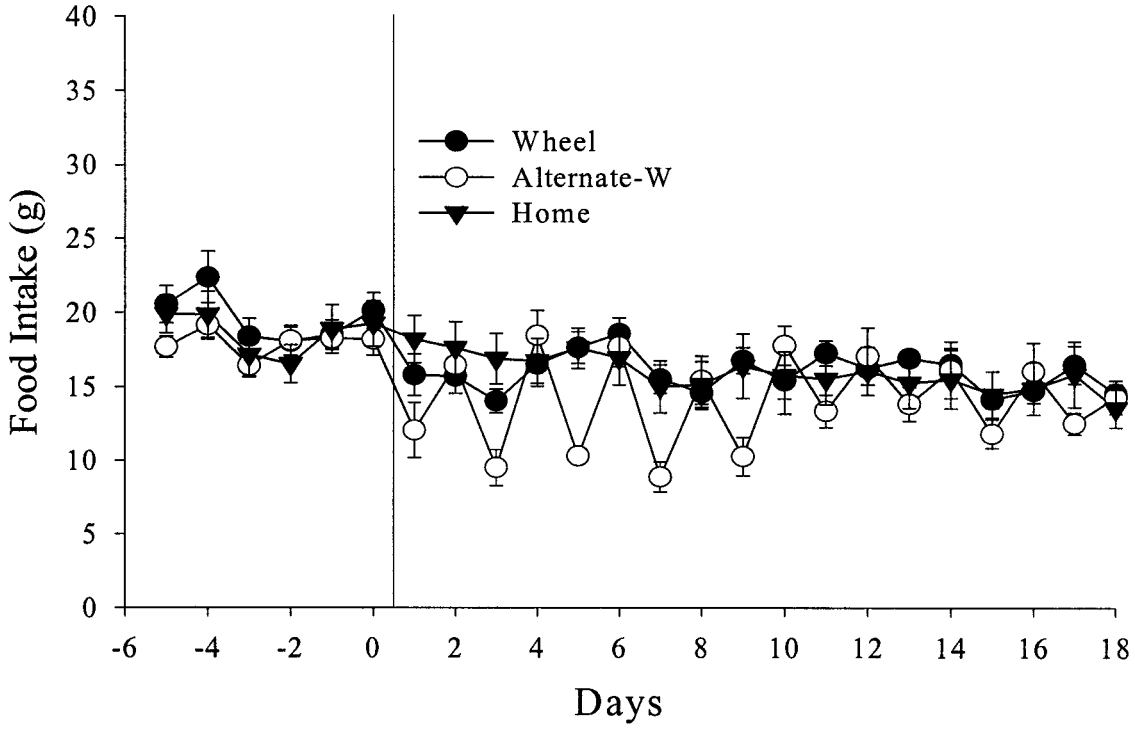


**FIGURE 7 – Sucrose Intake**

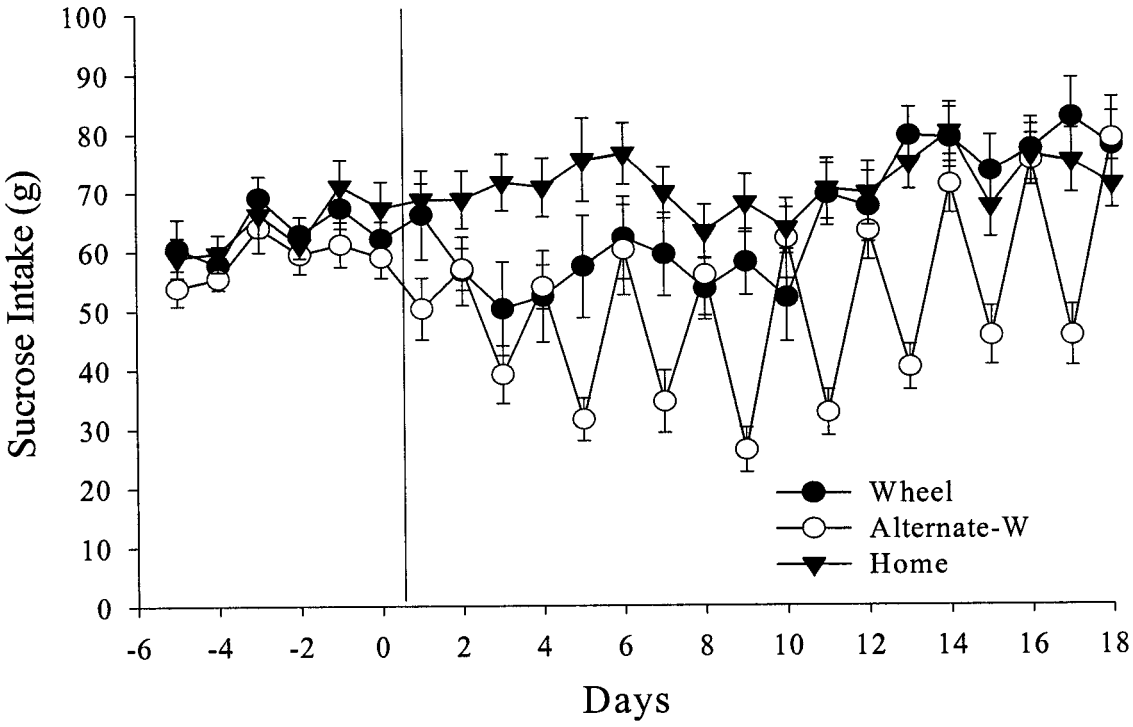
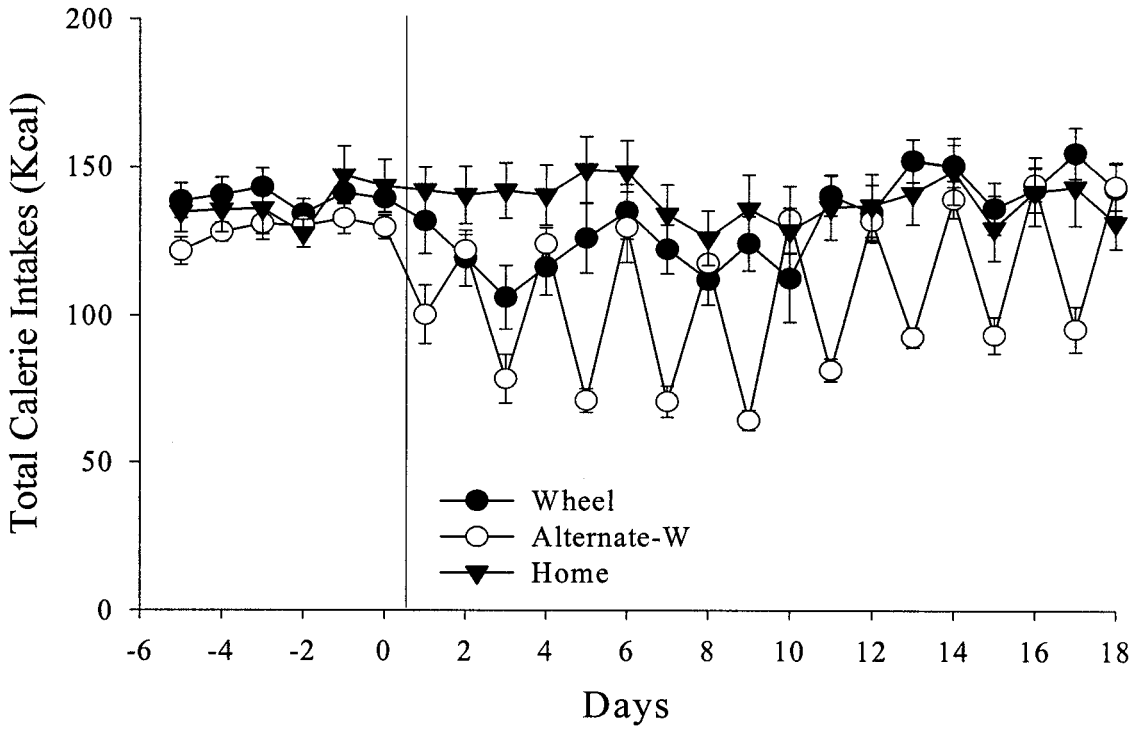


**FIGURE 8 – Total Calorie Intake**



## EXPERIMENT 3: FIGURES

**FIGURE 9 – Wheel Turns of Adults and Weanlings**

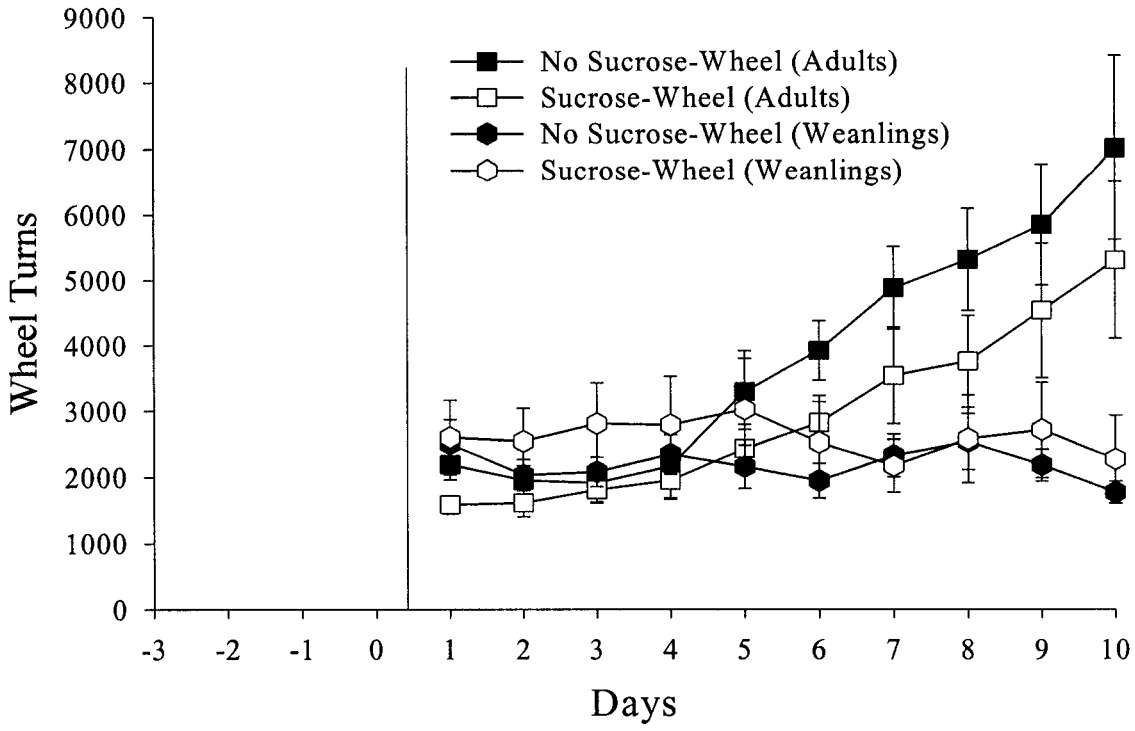
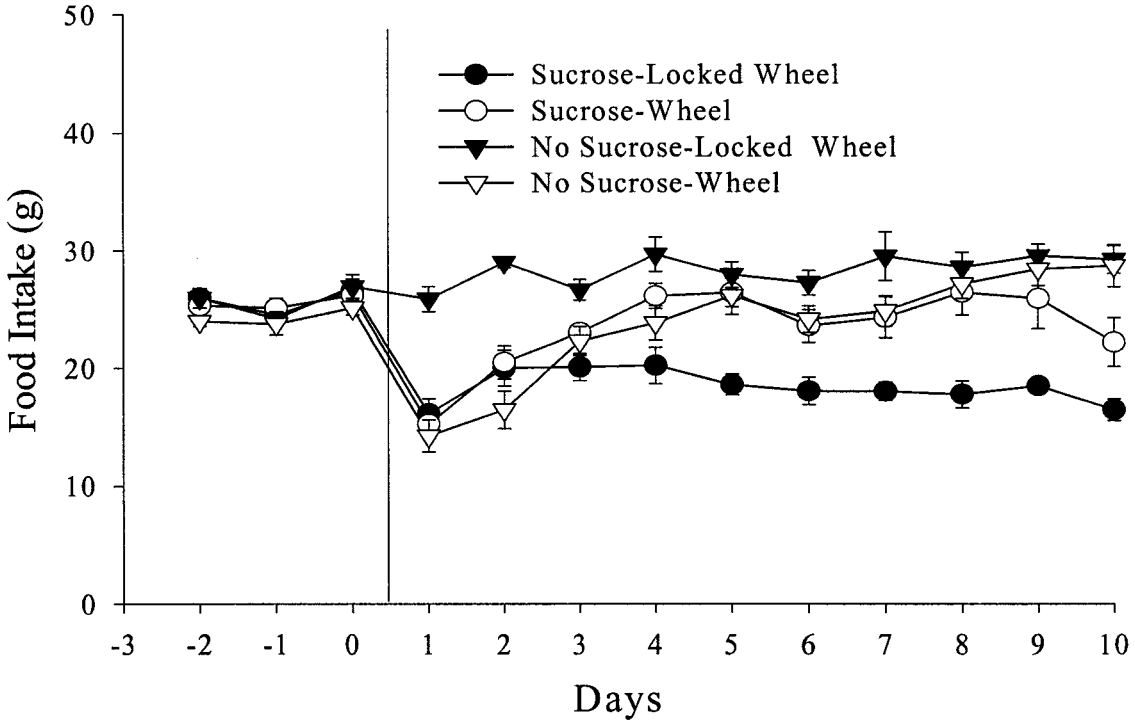


**FIGURE 10**

**A – Food Intake of Adults**




**B – Food Intake of Weanlings**

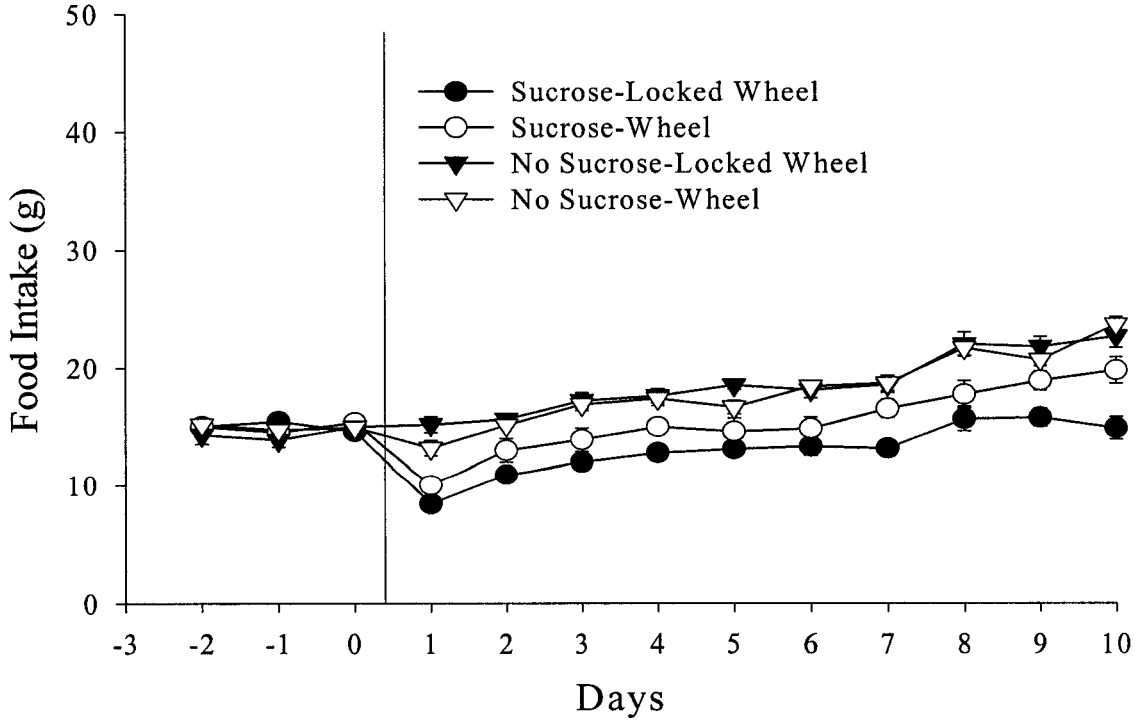


**FIGURE 11 – Sucrose Intake of Adults and Weanlings**

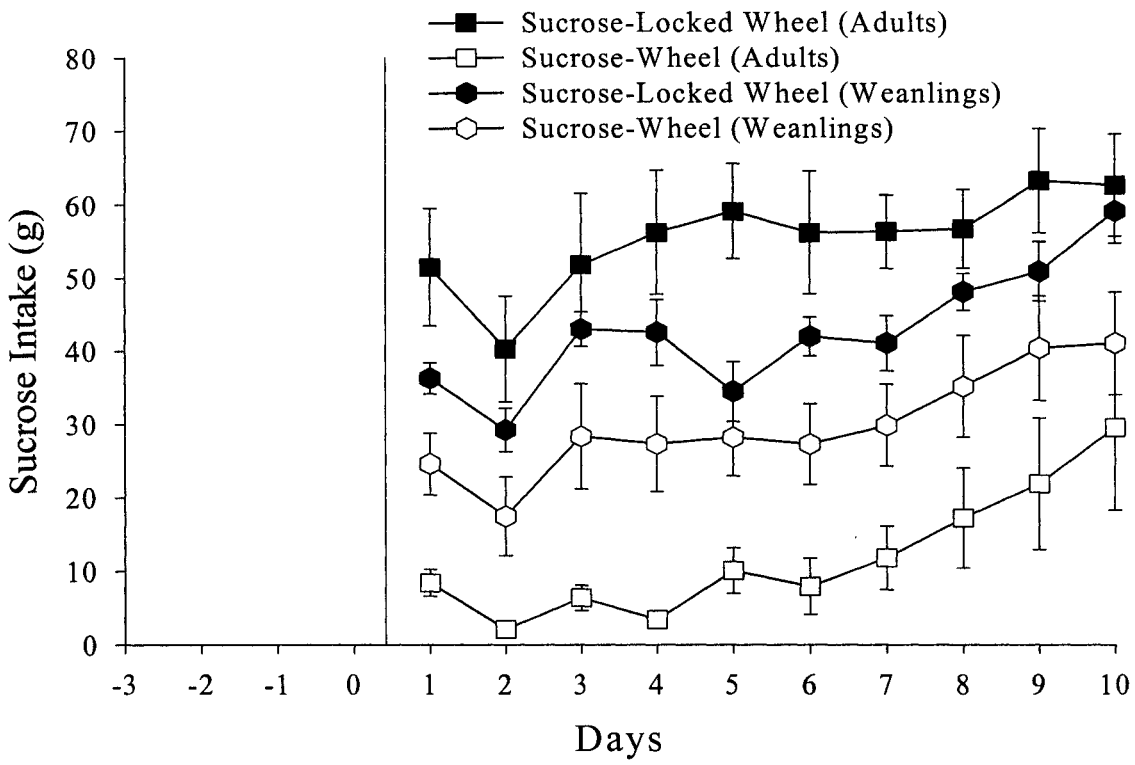
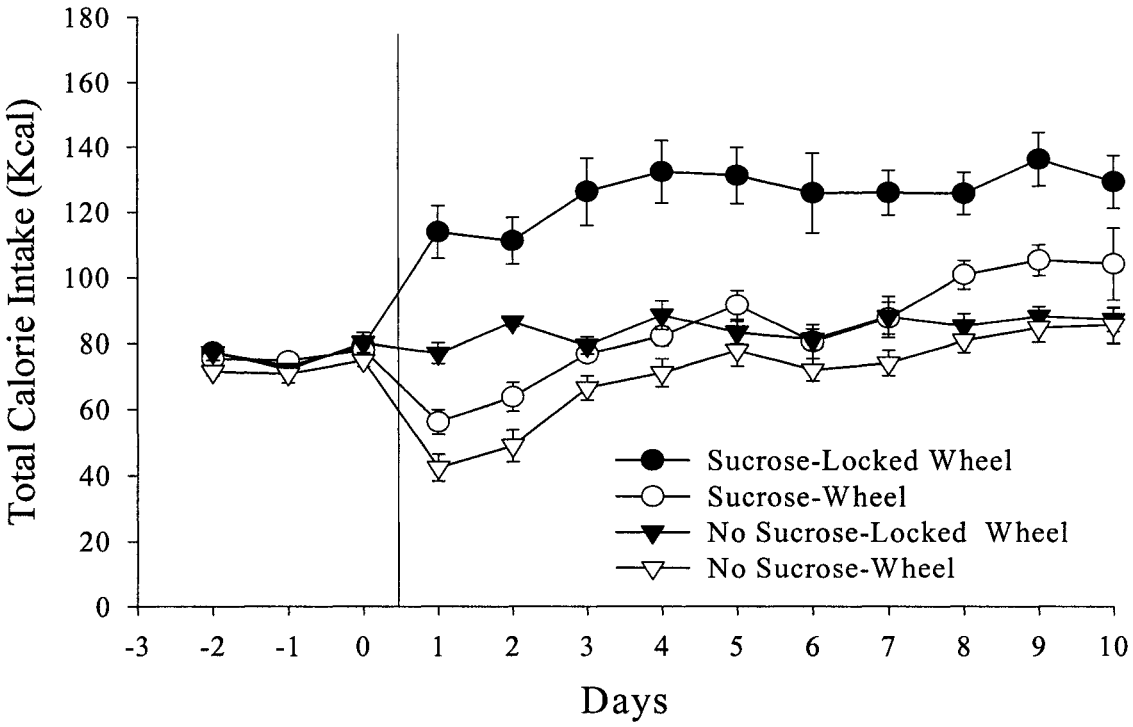


**FIGURE 12**

**A – Total Calorie Intake of Adults**




**B – Total Calorie Intake of Weanlings**