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Can Overeating Induce a Conditioned Taste Avoidance in Previously Restricted Rats?

by

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THESIS

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Abstract

While feeding is rewarding, the feeling of satiation has been theorized to be aversive under certain conditions. Using a food-restriction model of overeating developed in our laboratory the results presented here suggest that overeating, and the resulting experience of satiation, is capable of supporting a conditioned taste avoidance.

Rats had either ad lib (n=8) or restricted (n=24) food access (receiving 50% of the food eaten by ad lib fed rats) for 8 days. All rats were then given 24 hr access to a 0.1% saccharin solution, and two groups of food-restricted rats were given access to either 100% of the food eaten by the ad lib rats, or ad lib food access. This cycle was given once in Experiment 1 and three times (with 4 days between cycles) in Experiment 2. Rats were then returned to their previous feeding schedules for 1 day before being placed on ad lib food access until feeding was approximately equal for all groups. Rats were then tested for saccharin consumption in a two-bottle choice test of saccharin and water. Rats that were overeating at the time of the one-bottle saccharin acquisition trial(s) were expected to experience a change in internal state as a result of the influx in calories and associate this with the novel saccharin solution. This change in state should result in an avoidance of the saccharin at the two-bottle test.

In Experiment 1, at the two-bottle test, the restricted-ad lib feeding rats (those that overate) showed reduced saccharin consumption compared to the ad lib control rats, although these results were not significant. In Experiment 2, after three acquisition trials, the restricted-ad lib feeding group showed significantly reduced saccharin consumption compared to all other groups.

Experiment 3 sought to understand the learning component of the taste avoidance by making the saccharin familiar. Half of the rats were given 8 days of pre-exposure to the saccharin solution, after which they underwent the experimental procedure used in Experiment 2,

with half the rats on food restriction. Because they had previously shown the largest difference in saccharin consumption, only the rest-adlib (n=32) and adlib-adlib (n=32) groups were used in this experiment. After 8 days, all rats received 24 hr access to the saccharin solution, and the food restricted rats received access to ad lib food. Non-restricted rats received the saccharin without a change in feeding.

Saccharin consumption was measured and it was found that at the time of the two-bottle test, prior saccharin exposure attenuated the decreased saccharin consumption seen in the rest-adlib group in Experiments 1 and 2.

Overall, the results of these studies showed that the experimental design was successful in inducing overeating in previously restricted rats. When this bout of overeating was paired with a novel saccharin solution, rats later showed reduced consumption of the solution even after feeding levels returned to baseline. This reduced consumption was attenuated by pre-exposure to the saccharin solution.

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Introduction

While the act of feeding is generally considered to be a rewarding experience (food being the prototypical reward in learning and behaviour modification experiments) it is not completely evident that the sensation of satiety is also always rewarding. When people are very hungry (or are thoroughly enjoying a particular food) they will tend to eat a larger than normal amount, and then anecdotally report a feeling of malaise or gastrointestinal unease, suggesting satiety may be associated with a negative affect.

An examination of feeding in rats suggests this negative affect associated with satiety as well. When rats are completely deprived of food for a period of time, they are described as having an energy deficit. When these deprived rats are again given access to food they gorge on the first day, as might be expected, to compensate for this deficit. What is unusual is that on the days following this binge, they will sometimes show a "post-gorging behavioural low" during which they will consume less food, regardless of the remaining deficit (Lockard, 1967).

Similarly, in food restriction studies conducted in our laboratory (in which rats receive only half the normally eaten amount of food each day), they also show a pattern of binging when returned to ad lib food followed the next day by reduced food intake (Hertel, Botzang, Parfeniuk, & Eikelboom, In Press). Though this pattern of feeding is not always seen after food restriction, other studies using restricted food access have shown similar post-gorging reductions in consumption (Quimby, 1948; Armstrong, Coleman, & Singer, 1979; Harris & Martin, 1984; Gray, Fisler & Bray, 1988; Evans et al., 2005). Though most authors failed to comment on this bizarre feeding pattern, several did note the perturbations (Armstrong, Coleman, & Singer, 1979; Harris & Martin, 1984) and one commented on the possibility that this decreased intake may be evidence of a reaction or illness to the previous day's indulgence (Quimby, 1948).

These results highlight an important and ongoing debate in the literature regarding the nature of feeding, satiety, and the resulting intake of calories and nutrients. While some previous studies have argued that the influx of calories and nutrients is rewarding to rats and capable of producing a conditioned taste preference (Drucker, Ackroff, & Sclafani, 1994; Elizalde & Sclafani, 1990; Myers, Ferris, & Sclafani, 2005), there are aspects to satiety that appear to be aversive in nature. The post-gorging reduction in feeding, as seen in our, and others', research, may be evidence of this aversion.

Satiety

Satiety is a complex phenomenon involving a variety of interacting physiological factors working over both the short and long term. During meals, short-term satiety signals are generated in response to the type and amount of food being consumed (Smith, 1998). Long-term satiety signals may be more complex.

Numerous peripheral signals contribute to the regulation of food intake and energy homeostasis. Mechano- and chemo-receptors signalling the presence and energy density of food in the gastro-intestinal tract contribute to satiety in the immediate post-prandial period (Havel, 2001). GI chemo-receptors respond to the nutrient products of digestion, while mechano-receptors are activated by the entry of food into the stomach and small intestine. One well investigated signal is the duodenal peptide cholecystokinin (CCK). This signal was first shown to dose-dependently inhibit food intake in rats by Gibbs, Young, & Smith (1973a). The specific actions of this peptide have been further reviewed by Smith and Gibbs (1994) and Moran (2000). Additionally, GLP-1, ghrelin and gastrin-releasing peptide (GRP) also participate in the satiation process (Havel, 2001). Specifically, GRP appears to be involved in controlling the length of the intermeal interval (Rushing, Henderson, & Gibbs, 1998; Rushing & Gibbs, 1998).

Short-term meal induced signals, such as CCK and GRP, interact with long-term signals that are linked to the amount of fat stored in the body (Schwartz et al., 2000; Woods et al., 1998). The two most commonly discussed of these adiposity signals are the pancreatic hormone insulin and the adipose tissue hormone leptin. Increased levels of these hormones are associated with a positive energy balance. These long-term signals are hypothesized to adjust the sensitivity of the brain to the short-term meal generated signals (reviewed in Schwartz et al., 2000; Woods et al., 1998). In combination, these short- and long-term signals appear to influence the intake of food in organisms. When, for example an organism loses weight, less insulin and leptin are secreted and enter the brain, making it less sensitive to the meal termination signals. The individual therefore eats larger meals until body weight returns to normal levels. The reverse is true for rats that have gained weight, in that their brain becomes more sensitive to the short-term satiety signals resulting in smaller meals with longer time intervals between them.

There is some debate as to why satiety exists. In foraging rats that must work to get access to food, surely there would be a benefit to being able to consume large amounts of food at a single time, thereby reducing the energy required to search for and acquire food. However, it has been shown that when there are no restraints on feeding, rats will opt to consume a large number of small meals each day rather than a few large meals (Collier, 1989). This pattern of consuming many small meals suggests there may be costs associated with consuming a few large meals (Woods & Strubbe, 1994).

The Cost of Large Meals

It is known that the body is heavily guarded against over consumption, suggesting that there may be an associated cost. It has been shown that when food is consumed, especially large

meals, the influx of nutrients and calories perturbs many ongoing parameters that are closely regulated in the body, resulting in an internal change in physical state (Woods & Strubbe, 1994). Several of the body's homeostatically controlled systems are affected by this influx. As obvious examples, blood glucose and metabolic rate both increase during and after meals. To minimise the impact of these challenges, the body makes meal-anticipatory responses that lessen the magnitude of these meal-induced perturbations. Thus the levels of glucose in the blood (Campfield et al., 1996), as well as the ongoing metabolic rate (Even & Nicolaidis, 1985), decrease prior to meals. Additionally, cephalic phase insulin (insulin secretion that is initiated by the brain) is released at the time that humans or rats begin a meal. Insulin, in addition to reducing glucose levels, has been shown to reduce the levels of circulating lipids (Robertson et al., 2001), and to increase the levels of lipoprotein lipase (an enzyme that facilitates the removal of lipids from the blood; Picard et al., 1999).

Experimental evidence of the cost of large meals came from a series of experiments by Collier (Collier, 1986; Collier, 1989; Collier & Johnson 1990; Collier et al., 1986; Collier, Johnson, & Morgan, 1992) in which rats, after fulfilling an operant requirement, were given access to as much food as they could eat in a single meal. When the rats stopped eating (were sated) the food was removed and the rats had to perform the series of responses before the food was available again. This experiment differed from most previous operant conditioning experiments in that it put the operant cost at the level of the meal, rather than the individual food pellet. What the authors found was that when the cost of the meal was low (minimal work required to gain access to the meal), the rats would eat multiple small meals. However, as the cost of the meal increased, the rats would consume fewer, larger meals. These experiments highlight the expense of consuming large meals, in that consuming few, large meals is avoided unless the cost of the operant responses exceeds the costs of over consumption. While these

experiments point to the cost of over consumption, the nature of this cost was not apparent from these experiments.

One potential reason for these safe-guards against overeating may be a means to prevent the occurrence of hyperglycemia. Hyperglycemia, even when occurring only intermittently, is associated with a variety of physiological dangers. This is evidenced by the life long complications occurring in patients suffering from diabetes mellitus (e.g. Aoki et al., 2001), such as hypertension and increased risk of cardiac disorders, that are suggested to be the result of repeated and chronic heightened blood glucose levels. Because of the adverse consequences, it is suggested that this elevation of blood glucose would signal danger to an organism. There is behavioural evidence of this suggestion in that elevated glucose levels in the blood are sufficient to support the development of a conditioned taste avoidance (Deutsch, 1974).

Based on the above evidence, it is reasonable to assume that abnormally large meals could result in a (perhaps aversive) internal change in state in an organism. It is difficult to provide evidence for this suggestion, however, for several reasons. Firstly, there is the necessity and strong reward value of food itself. It has been theorized that when relatively small amounts of food are eaten, there is an abundance of positive after effects (such as the taste of the food). But, as the amount of food increases, the negative aspects (such as bloating, intestinal distension, and heightened blood glucose levels), assume more importance (Booth, 1985). This suggests that a relatively large meal must be consumed in order for a negative change in state to occur. Secondly, as mentioned previously, the body has developed a very complex system to control the intake of food in order to avoid the dangers associated with over-consumption. This system works partly in anticipation of food consumption. Even when large meals are consumed, if the animal is experienced with consuming this increased amount and can compensate for the consequences, then the adverse physiological effects are reduced.

Traditionally, when researchers are interested in demonstrating that an experimental manipulation is inducing a negative change in internal state, they will demonstrate this by utilizing a conditioned taste avoidance (CTA) paradigm. (The terms "conditioned taste avoidance" and "conditioned taste aversion" are used interchangeably in the literature. In this thesis the term "avoidance" will be used because it is more procedurally accurate and makes no assumptions about the internal affect associated with the procedure.)

Methodology for Assessing a State Change

One way to evaluate whether rats have experienced an internal state change as a result of an experimental manipulation is to use a CTA paradigm. The CTA paradigm involves pairing a novel tasting solution with the experimental stimulus (traditionally a toxic agent) in time, during one or many training sessions. There is then a period of time with no manipulations, followed by a reintroduction of the novel tasting solution. The amount of the novel solution consumed at reintroduction is then measured, allowing the experimenter to determine the degree to which the animal "avoids" the solution. This "test" phase can either be conducted by presenting the solution alone and measuring consumption (commonly referred to as a "one-bottle test") or using a more sensitive measure where the solution is presented along with a neutral fluid, such as water (commonly referred to as a "two-bottle test"). The CTA paradigm requires the animal to approach the bottle in order to sample the flavour; therefore it involves both an appetitive and consummatory phase of responding, and is a good measure of the motivation to consume the stimulus (Konorski, 1967).

Conditioned taste avoidance research traditionally centred on pairing novel tastes with known emetic agents (agents known to induce feelings of nausea). The typical illness inducing agent used in these studies is lithium chloride (LiCl; Berridge, Grill, & Norgren, 1981; Grill &

Norgren, 1978; Parker, 1982), though a wide variety of agents have shown similar conditioning abilities (reviewed in Parker, 2003).

In addition to known emetic agents, rats have also been shown to demonstrate CTAs to rewarding stimuli. Rats have been shown to avoid an amphetamine-paired flavour while simultaneously demonstrating a preference for a place paired with the drug (Reicher & Holman, 1977) and self-administering the drug (Wise, Yokel, & Dewitt, 1976). Similarly, voluntary wheel running is capable of supporting a CTA (Lett et al., 2001; Satvat & Eikelboom, 2006; Forristal, Hookey, & Grant, 2007), despite the finding that rats will work to gain access to a wheel (e.g. Iverson, 1993), and prefer places paired with wheel access (Lett et al., 2000; Lett et al., 2001).

An early theory, which attempted to explain paradoxical reports that reinforcing drugs produce taste avoidance, suggested that any novel change in state, even one that does not result in nausea, signals danger to the rat (e.g. Hunt and Amit, 1987). This is likely related to the inability of rats to vomit and rid their bodies of ingested substances, necessitating a highly sensitive internal response to the effects of ingesta. Because CTA paradigms are reflective of the internal state of rats, they are useful in investigating the effects of satiety and over-eating on the internal state of rats.

Satiety Signals and Cholecystokinin

Studies investigating the short-term satiety signal CCK attempted to induce satiety by administering the peptide exogenously. Gibbs, Young, and Smith (1973a) were the first to show that CCK could dose-dependently reduce feeding in rats when injected intraperitonially into fasted rats prior to food availability.

These studies suggested two competing theories on the method of action of CCK: The satiety hypothesis and the aversion hypothesis. The satiety hypothesis, first suggested by Gibbs

et al. (1973a) assumes that CCK acts specifically as a short-term satiety factor. In defence of their theory Gibbs et al. (1973a) cited the lack of evidence for a taste avoidance in their studies. The rats appeared neither ill nor hyperthermic, either of which would suggest that the rats were experiencing nausea as a result of the injection. The subjects also showed no evidence of a CTA when tested in a bait-shyness task. Later research showed that the administration of proglumide, a non-selective CCK antagonist, can increase food intake in rats when administered after a food pre-load, suggesting that in the absence of CCK, subjects stop showing behavioural signs of satiety (Shillabeer & Davison, 1982; 1985). Finally, CCK, when administered exogenously, not only inhibits feeding, but elicits the full behavioural sequence that has been described as accompanying satiety: the rats stop eating, engage in grooming or exploration for a short time, and then rest or sleep (Antin et al., 1975). The combination of these experiments suggests that CCK is at least partially necessary and sufficient to create the sensation of satiation in rats.

The alternative to the satiety hypothesis is that CCK is capable of inducing suppression in feeding because it is producing aversive internal cues (Deutsch & Hardy, 1977). This theory was originally posited when it was found that rats avoided flavours paired with CCK when given a choice in a two-bottle test (Deutsch & Hardy, 1977). Additionally, injections of CCK were shown to reduce operant responding for food (suggesting that the motivation to consume food is reduced; Maddison, 1977; Babcock, Livosky, & Avery, 1985), elicit pica in rats (ingestion of non-nutritive substances; McCutcheon, Ballard, & McCaffrey, 1992) and elicit defensive burying (rearrangement of cage bedding to bury a stimulus associated with an aversive internal state; Bowers et al., 1992). The results of these experiments mirror those seen in experiments using injections of LiCl, a known emetic agent (Mitchell et al., 1976).

The evidence investigating the nature of the effect of CCK was therefore found to be conflicting in that CCK appeared to be inducing satiety and aversion simultaneously. Eventually,

the suggestion arose that the idea that CCK reduces food intake either as a result of satiety or malaise may be too simplistic. Evidence began to appear that a component of satiety may in fact be some kind of aversive internal cue which terminates the motivation to feed (first discussed by Cabanac & LaFrance, 1990; 1991a; 1991b).

Sham-Feeding

The first evidence against the commonly held belief that satiety is rewarding and partially responsible for the rewarding nature of eating was a study by Van Vort and Smith (1983). The question raised by this study was whether hungry rats would learn to prefer a distinctively flavoured food that was both good tasting and satiating (real-fed) over a differently flavoured, but equally good tasting food that was not satiating (sham-fed). A sham-feeding paradigm involves the insertion of a fistula that diverts the food before it reaches the stomach. The animals therefore taste the food in their mouths, but never experience the after-effects. The results clearly demonstrated that hungry rats showed no preference between the real-fed and the sham-fed solutions, suggesting that satiety may not be a rewarding sensation.

In a later study, Van Vort and Smith (1987) trained food restricted rats to ingest a sweet milk diet that was flavoured with vanilla or almond extract during alternating real-feeding and sham-feeding sessions. On the first real-feeding session, one flavoured milk diet was available until the rat stopped eating and showed signs of satiation. On the following sham-feeding training day, the rat was given access to the same amount of the alternate flavoured milk diet and this cycle was repeated 17 times. Flavour preferences were assessed periodically by giving the rats 4-minute two-bottle choice tests between the real-fed conditioned stimulus (CSR) and the sham-fed conditioned stimulus (CSS). The researchers were attempting to determine whether rats would prefer the CSR, as would be expected if satiety was a rewarding sensation, or the CSS, as

would be expected if satiety was not a rewarding sensation. When rats were tested for their preference, the CSR accounted for only 33% of total fluid intake, suggesting they preferred the flavour not associated with the feeling of satiety.

In a related study, Warwick and Weingarten (1996) studied food-restricted rats that were trained to consume equal amounts of flavoured sucrose solutions in alternating real-feeding and sham-feeding sessions. Rats trained and tested with 24% sucrose solutions preferred the CSS solution in the first and only 30 minute two bottle test. Contrarily, rats trained with the 8% solution significantly preferred the CSR solution in a choice test. A third group trained and tested with 14% sucrose solution showed an intermediate preference.

Sclafani, Nissenbaum, and Ackroff (1994) examined the preferences of food restricted rats for real- and sham-fed solutions of different caloric concentration. Groups were trained to consume two differently flavoured 32% polycose solutions, one of which would be sham-fed. Two-bottle choice tests for the two solutions were conducted with CSR versus CSS under real and sham feeding conditions. In the first pair of two-bottle tests, the rats significantly preferred the CSS to the CSR; they were indifferent in the next three trials, but by the fifth test pair the rats preferred the CSR to the CSS. The CSR consumption changed from 29% in the first test to 69% in the last pair of tests. In marked contrast to these results, a second group that was trained with flavoured 8% (low caloric content) solutions significantly preferred the CSR solution from the first test. These results suggest that feeding, and the resulting influx of calories is rewarding to hungry rats when the caloric influx is small. However, when the rats were consuming a high-calorie solution, they seemed to find it less rewarding.

To explain these findings with respect to the rats' initial avoidance of the CSR when trained with concentrated solutions, it was proposed (Sclafani et al., 1994) that foods generate opposing post-ingestive feedback effects: a) positive reward signals that condition increased

flavour preferences and b) negative, i.e. satiating, stimuli that decrease flavour preferences. With concentrated nutrient sources, the negative feedback was hypothesized to outweigh the positive feedback so that the rats learned to avoid rather than prefer the CSR. The authors considered these positive and negative effects as an integrated process acting during acquisition of flavour preferences: the animal learns about the net reward value of flavours. They further proposed that with extended training, the rats adapted to the negative feedback signals (or the signals were reduced in intensity, i.e. stomach size increased) so that their initial avoidance changed to indifference or preference.

Distension of the Gastro-intestinal Tract

In addition to satiety signals such as CCK, afferent signals traveling in the vagal nerve fibres from stretch receptors in the stomach and proximal small intestine have been shown to be involved in the feeling of satiety as well (reviewed in Havel, 2000). As an alternative way to measure the aversive nature of satiety, Bardos (2001) demonstrated that intestinal distension is capable of producing a CTA. Bardos (2001) used internal distension via an implanted inflatable "balloon" in both the small and large intestinal loops to assess internal malaise and discomfort. The author used both isometric (increased tension without significant change in diameter), as well as volumetric (marked increase of gut diameter) distension. The rats were implanted with the balloon and allowed to recover before beginning the experimental sessions. After full recovery, the rats were water deprived for 23.5 hr and then given access to a flavoured saccharin solution; simultaneously the balloon was inflated to a point that the animal experienced mild discomfort. Four days later the rats were again given access to the flavoured solution and consumption was measured. It was found that rats which had first experienced the novel solution paired with the gut distension consumed less of the solution on the test days. This result was true

of both the volumetric and isometric distension. This study suggests that the stretching of the stomach that commonly accompanies the ingestion of food and satiety may be aversive to rats.

Experimenters also sought to examine whether satiety could be aversive using the taste reactivity (TR) test. Cabanac and LaFrance (1990; 1991a; 1991b) used rats fitted with gastric catheters that allowed the researchers to inject gastric pre-loads of various concentration and compensation (to satiate the rats) directly into their stomachs. The rats would show consummatory responses to a sweet sucrose solution infused across their tongue when hungry, but rejection reactions when they were first injected with the gastric pre-loads. These experiments suggested that once-palatable solutions became aversive when paired with the sensation of satiation.

Current Experiment

Taken together, the previous research in the field of satiety-induced CTAs lends convincing evidence to the idea that some components of food consumption and the following satiation may in fact be resulting in a potentially aversive internal state change. This area of research historically has relied on manipulations that may complicate the basic foundation of feeding behaviours. Techniques such as sham feeding, manual distension of the gastrointestinal tract and water deprivation fail to mimic the natural feeding behaviour of rats. It would be useful to investigate whether a CTA can in fact be produced in response to self-induced overeating in rats, as has been theorized to be possible (Woods, 1991). The post-gorging feeding behaviour discussed here may act as a model on which this theory can be tested.

When rats are placed on a food restriction diet for 8 to 10 days, they will overeat on the first day they are given ad lib access to food, eating approximately 125% of control consumption over 24 hr. This over-consumption may not be unusual given that the rats are very hungry and

are likely deficient in calories. What is unusual is that on the day following this overeating, the rats will often consume much less food than they had the previous day, followed by an increase in feeding on the third day following the binge before feeding levels gradually return to normal (e.g. Quimby, 1948). Using this food-restriction induced model of overeating developed in our lab, in which rats are restricted to half the normal amount of food eaten for 24 hr for a number of days, then given 24 hr of ad lib food access, these studies will attempt to determine whether this "post-gorging behavioural low" that occurs after the overeating day is a reaction to an internal state that results. Further, it will be investigated whether pairing this overeating with a novel tasting solution can support a CTA.

Traditional CTA paradigms have often utilized water deprivation as a means of tightly pairing the drinking of the solution with the presentation of the state change inducing agent (e.g Bardos, 2001). Because the current study uses overeating as an experimental manipulation, and because it is known that eating and drinking in rats are highly correlated behaviours (e.g. Kutsher, 1969), it would be ideal to use a CTA paradigm that does not require water deprivation.

Initial wheel running in rats has been known to produce a CTA in traditional CTA experiments (Lett & Grant, 1996; Lett et al., 2001). Forristall, Hookey, and Grant (2007) have demonstrated that when rats are water deprived and given access to a novel saccharin solution just before being forced or allowed to run on a running wheel for a limited period of time, they will display a CTA to the novel solution on subsequent presentations. This experiment used a traditional CTA design in that the access to the novel solution and state changing agent were tightly paired in time to ensure the rats associated the two. However, a strong CTA has also been demonstrated to wheel running in an experiment that did not use this tight temporal pairing. Satvat and Eikelboom (2006) gave rats access to familiar food, a novel sucrose solution and access to a running wheel, all on an ad lib basis for 24 hr periods. The experimenters found that

using this 24 hr design, the rats were still showing a complete suppression of the novel sucrose solution on subsequent presentations. Further, exposure to the novel sugar solution prior to wheel introduction was shown to attenuate the CTA (latent inhibition; introduced by Lubow and Moore, 1959; Lubow, 1973), suggesting that this paradigm is suitable for assessing the learning component of the avoidance. Ad lib access to a novel solution paired with wheel access, therefore, was able to produce a strong avoidance to the novel solution without limiting the access time of either the wheel or the novel flavour, but rather by presenting them simultaneously. Rats are able to associate a taste and illness over intervals of several hours (e.g. Revusky, 1968; Smith & Roll, 1967). Temporal continuity is less strict in CTA learning that in many other learning paradigms, which may explain the ability of this paradigm to support a CTA.

In the current experiment, using this 24 hr access design, rats will be given 8 days of food restriction followed by 24 hr of ad lib food access, a procedure found previously to induce overeating (Hertel, Botzang, Parfeniuk, & Eikelboom, In Press). Rats in this paradigm will generally eat 120%-130% the amount of food of ad lib control rats. By pairing this overeating with a novel 0.1% saccharin solution (a concentration shown to be the most highly preferred; Tordoff, Alarcon, & Lawler, 2008), it is hypothesized that an association should develop between the overeating and the taste of the solution. By then allowing the rats to return to normal feeding and retesting them several days later for the level of saccharin consumed compared to control rats, overeating rats should demonstrate an avoidance to the saccharin solution.

Experiment 1

Methods

Subjects. 32 (Experiment 1 & 2) or 64 (Experiment 3) male Sprague-Dawley rats (200-225 g, 47-50 days old at arrival) ordered from Charles River Canada were housed in individual polycarbonate shoebox cages and kept on a 12:12 light:dark cycle with lights on at 7:00 a.m. The mean temperature in the holding room was kept between 20° and 23°C. Rats received ad lib access to Harlan Techlad 8640 lab chow during the habituation period and ad lib access to water throughout the experiment. Rats were weighed daily at 12:00 p.m. The design of this and subsequent experiments were approved by the Wilfrid Laurier University Animal Care Committee, which follows the policies and guidelines of the Canadian Council on Animal Care (CCAC).

Procedure. (Outlined in Figure 1) Rats were habituated to the weighing and handling procedures for 8 days after arrival. All manipulations, handling and food and fluid changes occurred between 12:00 and 1:00 p.m. The experiment proper began on the ninth day after this habituation period and consisted of three phases.

Phase I (Days 1-8) of the experiment began with the rats being randomly assigned to one of two conditions. A group of 8 rats were kept on ad lib access to food while the remaining 24 rats began a food restriction schedule receiving 50% of the food consumed by the ad lib rats each day after their daily weighing, for 8 days.

Beginning at Phase II (on Day 9) of the experiment, 24 food-restricted rats were assigned to one of three new feeding schedules. For these rats, 8 remained on the restricted feeding schedule (Group rest-rest), 8 rats received the average amount of food consumed daily by the ad lib rats (Group rest-100%), and the final 8 rats received 24 hr of ad lib access to food (Group rest-adlib). The previously ad lib rats remained on ad lib food access (Group adlib-adlib). For

this day, all four groups also received ad lib access to a 0.1% saccharin solution in place of their normal water for 24 hr. Following the 24 hr saccharin exposure and the specified food availability, all previously restricted rats were returned to restricted feeding for 1 day (Day 10). This day was included to prevent the restricted rats from overindulging in the 24 hr following saccharin exposure and potentially developing an unintended association between the saccharin and overeating. All rats were then given 3 days of ad lib food access (Days 11-13).

Phase III (Day 14) of the experiment consisted of a two-bottle choice test. At this time all rats received a second 24 hr exposure to the 0.1% saccharin solution in addition to their water and ad lib food access. Throughout the experiment, food, water, and saccharin consumption was measured over each 24 hr period.

Results

The mean food consumption of rats in all groups over the complete experiment are shown in Figure 4, while their body masses are shown in Figure 5. Food consumption and body mass data from this experiment were analyzed for each phase of the experiment and provide the context for the saccharin consumption data.

Habituation (Days -3 to 0). For the habituation phase, a 4 (Days) x 4 (Groups) mixed Analysis of Variance (ANOVA) was performed on food consumption and body mass with repeated measures on the Day factor. Food consumption was stable across these days and similar for all groups, as seen in Figure 4. Body mass did not differ between groups over these 4 days (Figure 5) and as expected, all groups were gaining mass at a consistent and equal rate over days, $F(3, 84) = 669.9, p < 0.001$. The data of this phase of the experiment suggests that any differences between groups seen later are not due to pre-existing differences in feeding or body mass.

Phase I (Days 1-8). During this phase, rats in the three restricted groups were consuming a fixed amount of food (~15 g) each day. This phase was designed to induce hunger in the rats prior to the acquisition trial. Food data for restricted rats was not analyzed during this phase, but it is apparent from Figure 4 that there were no group differences in consumption. A repeated measures ANOVA of the 8 days revealed that ad lib rats were not increasing their consumption over these days.

Over this, and all subsequent phases, ad lib rats continued to gain mass (Figure 5). During the restriction phase restricted rats' body masses became increasingly reduced compared to the ad lib rats as seen in Figure 5. By the 8th day of restriction, a one-way ANOVA revealed significant group differences in body mass, $F(3, 28) = 167.3$, $p < 0.001$. Figure 5 shows no differences in body mass in the restricted groups.

Phase II (Day 9-13). A pre-planned independent samples t-test of the rest-adlib and adlib-adlib groups' feeding on the acquisition day revealed a significant difference in consumption between the two groups, $t(14) = 7.41$, $p < 0.01$. The other two groups were receiving fixed amounts of food, so this data was not analyzed.

Saccharin consumption on this day was analyzed using a one-way ANOVA and revealed a significant effect of group, $F(3, 28) = 6.61$, $p < 0.01$. Figure 6 shows (and a Tukey-B post hoc test confirms) that both groups that were experiencing a change in feeding at the time of the saccharin exposure (rest-100% and rest-adlib) and were consuming less saccharin than were the groups that did not experience a change in feeding (rest-rest and adlib-adlib) ($p < 0.05$).

After the acquisition trial, restricted rats were given 1 day of restricted feeding and then all groups were returned to ad lib access for 3 days. This phase was designed to return all groups' food consumption levels to approximately equal levels. The last day's food consumption of this period (Day 13) was analyzed using a one-way ANOVA and there were no significant

differences between groups (Figure 7). This suggests that prior to the two-bottle test; baseline feeding was similar in all groups.

A one-way ANOVA of the body mass data for the last day of Phase II (Day 13) showed significant group differences, $F(3,28) = 32.3$, $p < 0.001$. Figure 5 shows that the adlib-adlib group had the largest body mass, the rest-adlib and rest-100% fell in the middle, and the rest-rest group had the smallest body mass. The intermittent bouts of food access in the rest-100% and rest-adlib groups increased their body mass above that of the rest-rest group.

Phase III (Day 14). A one-way ANOVA of food consumption for Day 14 revealed no significant group differences in food consumption. This suggests that any differences seen in saccharin consumption are unlikely to be due to differences in food consumption. Though group body masses were still different at this time (Figure 5) this did not appear to affect saccharin consumption over the acquisition trials as the rest-rest and adlib-adlib groups that were the most divergent in body mass were consuming significantly similar amounts of saccharin. Body mass differences should therefore not have significantly affected saccharin consumption. It is also important to note that no groups experienced a change in feeding at the time of the two-bottle test. Any differences seen in saccharin consumption, therefore, were the result of conditioned and not unconditioned effects of the saccharin.

A one-way ANOVA of saccharin consumption on the two-bottle choice test day revealed no significant effect of Group. The rest-adlib group was still consuming less saccharin than were the remaining groups, however these differences were no longer significant, see Figure 7. These results suggest that while there were differences in consumption at the acquisition trial, these differences may have been eliminated as a result of the delay between the acquisition and two-bottle test.

Figure 7 also shows that water consumption was very low for all groups. A one-way ANOVA revealed no group differences in water consumption.

Discussion

This experiment tested whether overeating induced by a restricted food access schedule was capable of supporting a CTA to a novel tasting solution after a single acquisition trial. It is important to note that the food restriction procedure was successful in inducing overeating in the rats. Food consumption by the rest-adlib group was significantly higher than was food consumption in the adlib-adlib group at the time of the acquisition trial.

At the acquisition trial, both groups that were experiencing a change in feeding (rest-100% and rest-adlib) were showing significantly reduced consumption compared to groups that were not experiencing a change in feeding (rest-rest and adlib-adlib). These results were surprising considering previous research showing that restricted rats consume more saccharin than do their ad lib controls (Valenstein, 1967; Hursh & Beck, 1971). Acquisition trials were also used in Experiments 2 and 3 so an extended discussion of this phenomenon will be deferred to the general discussion section.

Food restriction was discontinued after the acquisition trial. Because drinking and feeding behaviours are known to be tightly linked (Kutscher, 1969), the two-bottle test was administered when all rats were consuming similar amounts of food. Food restriction also results in reduced weight, which may alter saccharin consumption. The two-bottle test was not deferred until body masses in all the groups were equal for two reasons: First, results from the acquisition trial suggest that differences in body mass did not necessarily result in differences in saccharin consumption. The two groups showing the greatest body mass difference (rest-rest and adlib-adlib) were consuming approximately the same amount of saccharin. Second, reintroduction of

the saccharin was done as soon after the acquisition trial as possible to prevent any forgetting.

At the two-bottle choice test the differences in consumption that were evident in the acquisition trial were no longer significant. While the overeating group was still consuming less saccharin than the remaining groups, the large variance in consumption resulted in non-significant results. Also, the rest-rest group had reduced their consumption at the two bottle test relative to their acquisition test consumption. Interestingly, the rest-100% group, which was consuming less saccharin than the rest-rest and adlib-adlib groups at the acquisition trial, was no longer showing reduced saccharin consumption.

A second experiment was designed to investigate further aspects of over-eating induced satiety. Repeated acquisition trials were scheduled in order to determine whether the results seen in the acquisition trial in Experiment 1 were the result of an initial change in feeding, or whether they would persist across multiple acquisition trials. Secondly, it was hoped that with repeated acquisition trials, the saccharin avoidance would be strengthened and resistant to the return to adlib feeding.

Experiment 2

Introduction

While robust and long-lasting reactions to novel taste stimuli after a single pairing of the novel taste and state-changing agent are possible (and common) in CTA paradigms, it has been demonstrated that repeated pairing of the novel taste with the state-change inducing agent can increase the strength of the resulting avoidance to the flavour (for example, Wolgin & Wade, 1990), or at least maintain a level of avoidance (for example, Siegel, Parker, & Moroz, 1995). For this reason, Experiment 2 used the paradigm from Experiment 1, with the exception that rats received repeated pairings of the overeating and saccharin prior to the two-bottle choice test. It

was expected that after repeated pairings of the overeating and the saccharin, the subjects would develop a stronger, longer lasting CTA that would remain significant in the two-bottle choice test.

Methods

Subjects. The rats (n=8 for each of the 4 groups) and handling procedure used here were the same as used in Experiment 1.

Procedure. (Outlined in Figure 2) The procedure for Experiment 2 was identical to that for Experiment 1 until the day following the first one-bottle acquisition session (Day 9). At this time, all previously restricted rats were returned to restricted feeding for 4 days (Days 10-13) followed by a second day of either 50%, 100% or ad lib food access paired with the saccharin solution (Day 14). This 4 day feeding cycle, with one-bottle saccharin availability associated with the increase in food availability, was repeated three times.

Following the third one-bottle acquisition (on Day 19), all restricted rats were returned to one day of restricted feeding before being given 3 days of ad lib feeding (Days 20-23). Then they were given a two-bottle water and saccharin solution choice test (Day 24).

Results

The mean food consumption of rats in all groups over the complete experiment are shown in Figure 8, while mean group body masses are shown in Figure 9. Food consumption and body mass data from this experiment were analyzed for each phase of the experiment and provide the context for the saccharin consumption data.

Habituation (Days-3 to 0). For the habituation phase, a 4 (Days) x 4 (Groups) mixed design ANOVA was performed on food consumption and body mass. Food consumption was

stable across these days and similar for all groups, as seen in Figure 8. Body masses did not differ between groups over these days (Figure 9) but as expected, all groups were gaining mass at a consistent and equal rate over days, $F(3, 84) = 280.1, p < 0.001$. These results suggest that any group differences seen in this experiment cannot be attributed to any pre-existing differences.

Phase I (Days 1 to 8). During this phase, rats in the restricted group were consuming a fixed amount of food (~15 g) each day. This phase was designed to induce hunger in the rats prior to the acquisition trials. Food data for restricted rats was not analyzed during this phase, but it is apparent from Figure 8 that there were no group differences in consumption. An 8 day repeated measures ANOVA revealed that ad lib rats were not increasing their consumption over days.

Over this, and all subsequent phases, ad lib rats continued to gain body mass (Figure 9). During the restriction phase, restricted rats' body masses while not explicitly decreasing was reduced compared to the ad lib rats (Figure 9). By the 8th day of restriction, a one-way ANOVA revealed significant group differences in body mass, $F(3, 28) = 81.4, p < 0.001$). Figure 9 also suggests there were no differences in body masses between the three restricted groups before the acquisition trials.

Phase II (Day 9 to 23). Over this phase, three acquisition trials were conducted with 4 days of restricted feeding (for previously restricted rats) between each. This procedure was designed to strengthen the avoidance seen in the acquisition trial in Experiment 1.

Three pre-planned independent samples t-tests were performed on the food consumption in the rest-adlib and adlib-adlib groups over the three acquisition trials. Each test revealed a significant difference in food consumption between the two groups (Day 9: $t(14) = 2.80, p < 0.05$; Day 14: $t(14) = 3.97, p < 0.01$; Day 19: $t(14) = 5.88, p < 0.001$)

Saccharin consumption over the 3 acquisition trials was analyzed using a 3 (Trials) x 4 (Group) mixed design ANOVA. There was a significant effect of Group on saccharin consumption over all three trials, $F(3, 28) = 10.61$, $p < 0.001$. Tukey-B post hoc tests revealed that the rest-adlib group was consuming significantly less saccharin than the rest-rest and adlib-adlib groups ($p < 0.05$), but not the rest-100% group. The rest-100% group was consuming less saccharin than the adlib-adlib group ($p < 0.05$). Figure 10 shows the consumption of the groups over the three acquisition trials. While the rest-adlib group consumption remained lower than the other groups, the reduced consumption in the rest-100% group appeared to diminish over the trials. There were no significant interactions, suggesting that consumption remained approximately stable over days.

After the acquisition trial, restricted rats were returned to one day of restricted feeding and then all groups were given ad lib access to food for 3 days. The goal of this manipulation was to make food consumption among all groups approximately equal before the two-bottle test. A one-way ANOVA of the last day of this phase (Day 23) found no differences in food consumption between groups (Figure 8). Therefore, prior to the two-bottle test, food consumption was approximately equal in all groups.

A one-way ANOVA of the body mass data for Day 23, showed significant group differences, $F(3, 28) = 36.4$, $p < 0.001$. Despite the fact that food consumption was now equal between groups, Figure 9 shows that the restricted groups were still reduced in body mass compared to the ad lib rats. Further, the rest-100% and the rest-adlib group rats had increased their body mass due to the intermittent increased food intake on the acquisition days.

Phase III (Day 24). A one-way ANOVA of food consumption on Day 24 confirmed that all groups were still consuming the same amount of food. This suggests that any differences observed in saccharin consumption cannot be attributed to differences in feeding. Though group

body masses were still significantly different (Figure 9) this did not appear to affect saccharin consumption over the acquisition trials as the rest-rest and adlib-adlib groups had the largest body mass difference but were consuming significantly similar amounts of saccharin. Differences in body mass should, therefore, not drastically affect saccharin consumption on this two-bottle test day. Procedurally no groups were experiencing a change in feeding at this time and it is therefore assumed that any difference in saccharin consumption at the two-bottle test was the result of conditioned and not unconditioned effects.

A one-way ANOVA of saccharin consumption on the two-bottle choice test day revealed a significant effect of Group, $F(3, 28) = 7.0, p < 0.001$. Tukey-B post hoc analyses revealed that the rest-adlib group was consuming significantly less saccharin than were the other three groups (see Figure 11). Interestingly, the rest-100% group that had reduced consumption across the acquisition trials now showed consumption levels similar to the groups that never experienced a change in food consumption. It appears that when the rest-100% group was not experiencing a change in food consumption at this two-bottle saccharin test, they no longer showed reduced saccharin consumption.

Figure 11 also shows water consumption at the two-bottle test for all groups. Levels of consumption were very low in all groups and a one-way ANOVA revealed no group differences in consumption. This suggests that even though the rest-adlib group rats were drinking relatively less saccharin than were the remaining groups, they still preferred the saccharin to the water.

Discussion

This experiment was designed to address several questions that were raised following the examination of the results from Experiment 1. The acquisition trial in Experiment 1 led to unexpected results in that both groups that experienced a change in feeding consumed less

saccharin, though this effect was not evident at the two-bottle test after feeding had returned to normal. Experiment 2 sought to determine whether the reduced consumption in the groups experiencing a change in feeding would persist across multiple trials. Second, at the two-bottle test in Experiment 1 data were suggestive of a CTA in that the rest-adlib group was consuming less saccharin than the remaining groups, but this difference was not significant. Experiment 2 explored whether multiple acquisition trials would produce a stronger avoidance that would be evident after food consumption had returned to baseline levels.

As in Experiment 1, the 8 day food restriction paradigm and the 4 day gaps between acquisition trials were successful in inducing overeating in the restricted rats. At all acquisition trials, the rest-adlib rats were consuming significantly more food than were the adlib-adlib rats. These data suggest that if there was no avoidance to saccharin at the final test, it could not be attributed to a lack of over-eating.

Over all three acquisition trials, results were similar to those seen in Experiment 1. The rest-adlib rats were consuming significantly less saccharin than the two groups that were not experiencing a change in feeding (rest-rest and adlib-adlib); however, here the rest-100% group was only consuming significantly less saccharin than the rest-rest group. Figure 10 suggests that the effects of the change in feeding in the rest-100% group were decreasing over trials as their saccharin consumption converged with that of the rest-rest group. However, the rest-adlib group maintained a consistently reduced consumption level across trials.

After the three acquisition trials, all previously restricted groups were given 1 day of restricted feeding again in order to prevent any learning that may have occurred by the rest-rest and rest-100% groups having ad lib food available immediately after the last saccharin exposure. After this day, all groups were returned to ad lib feeding. This manipulation was meant to achieve equal food consumption among all groups before the two-bottle saccharin test and was

successful. Figure 8 shows that all groups were consuming approximately equal amounts of food at the time of the two-bottle test.

The results of the two-bottle test were as hypothesized. The rest-adlib rats were the only rats consuming significantly less saccharin than rats in the remaining groups. (The reduced saccharin consumption seen in the rest-100% group over the acquisition trials was no longer evident.) This experiment demonstrated that a change in feeding was capable of reducing consumption of a novel solution. However, when feeding was returned to normal and did not change when the novel solution was available, only a drastic overeating was capable of supporting a conditioned avoidance.

Experiment 3

Introduction

In order to better understand the overeating avoidance relationship and to support the argument that the decreased saccharin consumption seen in Experiment 2 in the rest-adlib rats was the result of a learned avoidance, Experiment 3 sought to demonstrate that prior exposure to the novel tasting solution will attenuate the avoidance due to latent inhibition (Lubow & Moore, 1959; Lubow, 1973). The novelty of a taste is an important aspect of the CTA paradigm. Pre-exposure to the novel taste prevents the taste from being associated with the change in state brought on by the experimental manipulation (see: Best, 1975; Nakajima & Nagaishi, 2005; Heth & Pierce, 2007). This is believed to occur because the rats perceive the taste as not being harmful after repeated exposures to the taste with no concurrent changes in their internal state. Therefore when the change in internal state occurs, it is unlikely that the rats will associate this change with the now safe taste. This third experiment sought to attenuate the CTA seen in Experiments 1 and 2 in the overeating rats to the saccharin solution by giving half the rat's prior exposure to the

solution without pairing it with overeating. A group of non-overeating control rats was also given the same pre-exposure to determine how this affects saccharin consumption.

Methods

Subjects. The rats (n = 16 in all 4 groups) and handling procedure used here were the same as that used in Experiment 1 and 2.

Procedure. (Outlined in Figure 3) Phase I of the experiment began with the rats being randomly assigned to one of two conditions (exposure factor). Half of the rats were given 8 days (Days -7 to 0) of continuous access to a 0.1% saccharin solution in place of their water (exposed; "e" groups). The remaining rats were given water only (no prior exposure). After the 8 days all rats were returned to only water access.

Phase II (Days 1 to 8) of the experiment involved randomly assigning the exposed ("e") and non-exposed rats into one of two feeding schedules (restriction factor): half of the rats remained on an ad lib feeding schedule ("adlib") while the remaining 16 rats were placed on a restricted feeding schedule ("rest"), receiving 50% of the food consumed daily by the ad lib rats. This phase lasted for 8 days.

Phase III (Days 9-23) of the experiment involved repeated pairings of one-bottle acquisition trials of the saccharin solution with adlib access to food for all groups for 24 hr. This resulted in there being four experimental groups; the rats with pre-exposure to the saccharin and adlib or restricted food (groups "e-adlib-adlib" and "e-rest-adlib", respectively) and the rats without pre-exposure to the saccharin and adlib or restricted food (Groups "adlib-adlib" and "rest-adlib", respectively). These acquisition trials were separated by 4 days of restricted feeding for the restricted groups. Following the third acquisition trial, the restricted rats were returned to

restricted feeding for 1 day (Day 20) followed by 3 days of ad lib food access (Days 21-23) until all groups were eating equal amounts of food.

Phase IV (Day 24) of the experiment involved giving all the rats a two-bottle choice test of saccharin and water with ad lib food access.

Results

The mean food consumption of rats in all groups over the complete experiment are shown in Figure 12, while mean group body masses are shown in Figure 13. Food consumption and body mass data from this experiment were analyzed for each phase of the experiment and provide the context for the saccharin consumption data. All statistics were run using exposure (exposed or not exposed to the saccharin solution in the exposure phase) and restriction (food restricted or not food restricted during the food restriction phase) factors to compare the four groups.

Habituation (Days -11 to -8). For the habituation phase a 4 (Days) x 2 (Restriction) x 2 (Exposure) mixed design ANOVA was performed on food consumption and body mass. Food consumption was stable across these days and was similar for all groups, as seen in Figure 12. Body mass did not differ between groups over these 4 days (Figure 13). As expected, all groups were gaining mass at a consistent and equal rate over days, $F(3, 177) = 557.6, p < 0.001$. These results suggest that any differences seen in this experiment are un-likely to be due to pre-existing differences.

Phase I (Days -7 to 0). An 8 (Days) x 2 (Restriction) x 2 (Exposure) mixed design ANOVA was performed on body mass and food consumption for the saccharin pre-exposure phase. Both analyses revealed only a significant effect of Days, $F(7, 420) = 820.1, p < 0.001$ and $F(7, 420) = 5.4, p < 0.001$, respectively. These results suggest that over these days all groups

were gaining body mass at a consistent and similar rate and were showing day to day differences in food consumption. It is important to note that saccharin consumption over this pre-exposure stage did not affect food consumption or body mass gain.

In this phase half of the rats were given 8 days of saccharin pre-exposure to familiarize them with the solution (Figure 14). Saccharin consumption in this phase was analyzed using an 8 (Days) x 2 (Restriction) ANOVA. There was no significant effect of Restriction on saccharin consumption. This suggests that any subsequent differences in saccharin consumption were not due to pre-existing differences between groups. It is also important to note that there was no significant effect of days, suggesting that saccharin consumption was not changing over days. This suggests that subsequent differences in saccharin consumption were not due to an increased consumption from the exposure phase.

Phase II (Days 1 to 8). The two restricted groups were consuming a fixed amount of food (~15 g) at this time so the data for this phase were not analyzed for these groups. It is apparent from Figure 12 that there were no differences in food consumption in these restricted groups. Food consumption in the ad lib groups was analyzed using an 8 (Days) x 2 (Exposure) mixed design ANOVA. Both groups showed day to day variance in their food consumption, $F(7, 210) = 2.2, p < 0.05$. Figure 12 demonstrates that these two ad lib groups did not differ in their consumption over this phase.

Over this, and all subsequent phases, ad lib fed groups continued to gain body mass at a consistent and equal rate (Figure 13). During this phase, as expected, the restricted groups failed to gain body mass resulting in a significant difference in body mass between the restricted and ad lib groups by Day 8, $F(1,60) = 15.1, p < 0.001$. Figure 13 shows that both restricted groups and both ad lib groups were approximately equal in their body masses. By the end of Phase II, restricted rats weighed approximately 81% of the ad lib rats (Figure 13).

Phase III (Day 9 to 23). Food consumption on the acquisition trials was analyzed using a 3 (Days) x 2 (Restriction) x 2 (Exposure) mixed design ANOVA. There was a significant effect of the restriction, $F(1, 60) = 18.87, p < 0.001$, and an Exposure x Restriction interaction, $F(1, 60) = 4.51, p < 0.05$. These results suggest that the restriction paradigm was successful in inducing overeating in the restricted groups when they had ad lib food access. It is apparent from Figure 12 that the degree of overeating appeared consistent across the three exposure trials. This suggests that the rats were overeating to the same degree on each of the acquisition trials. Therefore, any change in saccharin consumption across the acquisition trials was not the result of a decline in the level of overeating in the previously restricted rats. Further, Figure 12 suggests that the levels of feeding within the two restricted and two ad lib fed groups were not different but that the interaction may be due to the small differences between the exposed and non-exposed conditions in the two feeding conditions.

Saccharin consumption over the acquisition trials, shown in Figure 15, was analyzed using a 3 (Days) x 2 (Restriction) x 2 (Exposure) mixed design ANOVA. As was observed in previous experiments, there was a significant effect of Restriction, $F(1, 59) = 13.83\ p < 0.001$. (Due to spillage, data from one animal in the adlib-adlib group was lost resulting in 15 rats in this group.) Figure 15 shows that the rest-adlib rats were consuming significantly less saccharin than the adlib-adlib rats over the three acquisition trials. However, in this ANOVA there was also a Restriction x Exposure interaction which approached significance, $F(1, 59) = 3.381, p = 0.07$. It is apparent from Figure 15 that the rest-adlib group was consuming less saccharin than were the remaining groups across all trials but averaged over the three trials it appears the e-rest-adlib group was not as different from either of the adlib groups. The nearly significant interaction suggests that while prior exposure did not affect saccharin consumption under normal conditions

(in the two adlib groups), but it did appear to attenuate the reduced consumption in the two rest groups.

On the last day of ad lib food access before the two-bottle test (Day 23) the 2 x 2 between groups ANOVA revealed there were no differences in food consumption between the groups (Figure 12). These results suggest that the 3 days of ad lib feeding were sufficient to produce similar food consumption across all groups. A similar ANOVA found there was no longer an effect of restriction on body mass (Figure 13). This is possibly the result of the increased age of the rats resulting in decreased daily body mass gain in the adlib rats. Any differences in saccharin consumption at the two-bottle test cannot be attributed to group differences in body mass or food consumption.

Phase IV (Day 24). A 2 x 2 between groups ANOVA of food consumption on Day 24 revealed no group differences. The restricted rats were now consuming an approximately equal amount of food as the ad lib rats (Figure 12). These results suggest that any differences seen in saccharin consumption on this day were not the result of different levels of food consumption between groups.

Saccharin consumption at the two-bottle test was analyzed using a similar 2 x 2 between groups ANOVA and a significant effect of Restriction, $F(1, 60) = 8.5, p < 0.01$, as well as a significant Restriction x Exposure Interaction, $F(1, 60) = 11.6, p < 0.01$ were found. Figure 16 shows that the rats given saccharin pre-exposure were consuming more saccharin than the non-exposed rats in the restricted groups, but this trend was reversed in the ad lib groups, with the non-exposed rats consuming more saccharin than the previously exposed rats. The two groups exposed to saccharin previously had similar consumption in this two-bottle test at the end of the experiment. Prior saccharin exposure attenuates the reduced avoidance that occurs when saccharin exposure is paired with a change in feeding schedule. This suggests that the reduced

saccharin consumption seen in the current and previous experiments is the result of conditioning, as it does not occur when the paired solution is no longer novel. As in Experiments 1 and 2, water consumption was very low and comparable in all groups.

Discussion

This experiment was designed to further investigate the learning component of the reduced saccharin consumption that was observed in Experiments 1 and 2, both at the acquisition trial(s) and at the two-bottle tests. Using a latent inhibition paradigm (Lubow and Moore, 1959; Lubow, 1973), half of the rest-adlib and of the adlib-adlib groups (the groups showing the greatest differences in saccharin consumption in the two-bottle tests in Experiments 1 and 2), were given exposure to the saccharin solution prior to the experimental procedure. It was expected that if the reduced consumption observed in the groups experiencing a change in feeding was the result of a learned association between the overeating and the saccharin exposure, then familiarity with the saccharin should attenuate this reduction.

Saccharin consumption in the pre-exposure phase was consistent across days. This was beneficial in that it seems that prior exposure does not increase saccharin consumption on later exposures per se. This suggests that any differences in saccharin exposure observed in either the acquisition trials or the two-bottle test were not due to exposure increasing consumption under normal feeding consumption. It should, however, be noted that consumption on an ad lib feeding schedule was lower than that found when rats were exposed to saccharin approximately every 5 days. In other experiments the same difference in consumption for continuous and discontinuous access to sweets has been observed (Hewitt, 2007 honours thesis).

It is also useful to note that, as in previous studies, food restriction was successful in inducing overeating in the rats. Food consumption in the rest-adlib groups was significantly

higher across all acquisition trials than consumption in the adlib-adlib groups. Figure 15 shows that the level of food consumption was consistently high across acquisition trials in the rest-adlib group. This suggests that any changes in saccharin consumption over the three trials were not the result of changes in overeating.

Saccharin consumption over the three acquisition trials, shown in Figure 15, was significantly reduced in the rest-adlib group. The reduced consumption in this group was stronger than that in the e-rest-adlib group suggesting prior saccharin exposure weakened the unconditioned effects seen with overeating.

The results of the two-bottle test suggest that there was a significant interaction between restriction and prior saccharin exposure. Only in the non-exposed deprived rats was the overeating able to induce saccharin avoidance. It is apparent from Figure 16 that while prior saccharin exposure increased saccharin consumption in the rest-adlib group compared to their non-exposed controls, the effect was opposite in the adlib-adlib groups. These results are consistent with the hypothesis that the reduced saccharin consumption seen in the rest-adlib groups was the results of a conditioned association between the overeating and the saccharin. When rest-adlib rats were given previous exposure (group e-rest-adlib) with the saccharin solution, they no longer associated the saccharin with the overeating and as a result, no longer showed reduced consumption.

General Discussion

The aim of this thesis was to provide behavioural evidence that satiety caused by overeating in rats could support a CTA to a novel tasting solution when the two are paired utilizing a 24 hr ad lib access design. Satiety-induced taste avoidance has been found in previous studies, though these experiments have typically used unnatural protocols such as sham feeding

(e.g. Van Vort & Smith, 1983, 1987) or manual distension of the gastrointestinal tract (e.g. Bardos et al., 2001), in concert with restricted water access. This thesis attempted to add to the findings of these past experiments using a more natural behavioural protocol of self-induced overeating.

Previous research in our laboratory has shown what is described as a "post-gorging behavioural low" (Lockard, 1967) in which rats show reduced food consumption the day following the ad lib binging that occurs after food restriction (Hertel, Botzang, Parfeniuk, & Eikelboom, In Press). This phenomenon has been found in previous studies (Quimby, 1948; Armstrong, Coleman, & Singer, 1979; Harris & Martin, 1984; Gray, Fisler, & Bray, 1988; Evans et al., 2005), though has not (to my knowledge) been thoroughly examined. The post-gorging behavioural low seen in these previous experiments may be the result of a, possibly aversive, internal state change resulting from the previous days over consumption. The experiments presented here attempted to pair this state change with a novel tasting solution, and assessed whether this pairing could support a CTA.

Using a traditional CTA design in which the experimental manipulation and novel tasting solution were tightly paired in time was not practical. Previous research, such as the study by Bardos et al. (2001), used water restriction to ensure consumption of the novel tasting solution when the state change inducing agent was introduced. Use of water restriction in our experiments was not practical because it is difficult to induce overeating in rats that are water restricted, as the two behaviours are naturally correlated (Kutscher, 1969).

Additionally, food consumption in restricted and ad lib fed rats takes on a very different pattern. When restricted rats are given ad lib access to food, they will consume the majority of their daily food immediately (unpublished observations), whereas continuously ad lib fed rats favour consuming many smaller meals over a longer period of time (Collier, 1989). Therefore,

creating time restraints on consumption to allow for adequate pairing of the novel taste and eating in these two groups would be difficult.

A non-traditional CTA design using 24 hr pairing of the experimental manipulation and novel taste was previously used by Satvat and Eikelboom (2006). Ad lib wheel access and ad lib sucrose access were both given for several days and a long-lasting, complete avoidance to the sucrose developed. A similar procedure was used in the current experiments to induce a conditioned taste avoidance without restricting water access or food availability in ad lib fed rats.

To ensure overeating at the time of the acquisition trials, rats were maintained on a restricted food access schedule. This manipulation was successful in inducing overeating in the rest-adlib group rats in all experiments. For all the acquisition trials, the rest-adlib rats were consuming significantly more food than were the adlib-adlib rats. Thus, if there were no differences in saccharin consumption at the acquisition or two-bottle tests, it was not due to the absence of overeating.

Saccharin consumption over the acquisition trials in all three experiments produced some unexpected results. In Experiment 1 (Figure 6) and Experiment 2 (Figure 10), it was found that over the one or three acquisition trials, all rats that experienced a change in feeding (rest-100% and rest-adlib rats) showed reduced saccharin consumption compared to those groups that were maintained on a consistent feeding schedule (rest-rest and adlib-adlib rats). These results were unexpected in that previous research has shown that hungry rats will consume more of a sweet solution relative to satiated controls (e.g. Valenstein, 1967; Hursh & Beck, 1971). This would suggest that the rest-rest group should have been consuming the largest amount of saccharin, but the results show that the rest-adlib and rest-100% groups were consuming less saccharin than were the rest-rest groups which in turn were consuming amounts similar to the adlib-adlib groups.

These results may be an artefact of the 24 hr, ad lib access design of the experiment. Restricted rats were given access to the food and saccharin at the beginning of the 24 hr period. Restricted rats will generally consume the majority of their food immediately after it is given (unpublished observations) meaning that they may have overeaten early in the night and then avoided the saccharin for the remainder of the 24 hr.

Experiment 3 involved a pre-exposure to saccharin phase prior to the food restriction phase in the experiment that was useful in examining this decreased consumption in the groups experiencing a change in feeding. In the pre-exposure phase, half of the adlib-adlib and half of the rest-adlib groups received 8 days of saccharin exposure to familiarize them with the solution. At the time of the acquisition trials, the saccharin naïve rest-adlib rats showed reduced saccharin consumption (Figure 15) as in Experiment 1 and 2. However, the e-rest-adlib rats (those that were pre-exposed to the saccharin solution) did not show this avoidance, and consumed saccharin at rates comparable to both the adlib-adlib and e-adlib-adlib groups. Therefore, it is assumed that the decreased saccharin consumption seen in the acquisition trials of all three experiments in the feeding changed rats was in fact the result of an avoidance developing early in the 24 hr acquisition phases, as this avoidance was attenuated when the saccharin solution was not novel.

The results of the acquisition trials, specifically those of Experiments 2 and 3, provide convincing data for the hypothesis that overeating can support a CTA. Both groups that were experiencing a change in feeding (and could have been overeating at food introduction) were showing reduced saccharin consumption compared to groups that were not overeating. It was not clear at this point, however, whether this avoidance found in the acquisition trials would persist when the saccharin availability was no longer paired with the bout of overeating.

The two-bottle tests from Experiments 1 and 2 show that the avoidance that was observed in the rest-100% group may have been the result of an unconditioned effect of the change in feeding resulting in an acute neophobia, as this group did not show the avoidance at the two-bottle test once food availability was returned to baseline levels, and the saccharin was no longer given concurrently with a change in feeding. Familiarity with saccharin would also reduce this neophobia as was found in Experiment 3.

It is interesting to note that the avoidance observed in these acquisition trials may not have been the result of overeating *per se*, but rather a result of a change in feeding schedule. Because the rest-100% group failed to show an avoidance to the solution at the two-bottle test in both Experiments 1 and 2, it is suggested that the decreased consumption seen during the acquisition trials may have been due to a change in food availability. For this reason, it is plausible to hypothesize that other changes in feeding schedule, even one that introduces restricted food access to previously ad lib fed rats, should produce a comparable decline in saccharin consumption. This proposed experiment would of course be subject to rigorous control conditions, as restricted food access is known to affect fluid consumption (Kutscher, 1969).

The results in Experiment 1 were not entirely clear with regard to the hypothesis that overeating induced satiety would support a CTA. The difference in consumption between the rest-adlib group and the remaining groups, while present at the two bottle test, was not significant. This may have been the result of a weak avoidance that resulted from only a single pairing of the overeating and the saccharin.

In Experiment 2, with three acquisition trials, the rest-adlib group showed saccharin avoidance at the two-bottle test that was as pronounced as that seen in the acquisition trials. These results confirm that rats were learning an association between overeating and saccharin as they continued to avoid it even after overeating was no longer occurring.

Similarly, in Experiment 3, the results of the two-bottle test were consistent with a CTA hypothesis. Prior saccharin exposure prevented the avoidance. Figure 16 shows that in the adlib-adlib groups, saccharin consumption may have decreased slightly as a result of pre-exposure. In the rest-adlib groups however, pre-exposure resulted in saccharin consumption at levels similar to that observed in the adlib-adlib groups suggesting a latent inhibition for the CTA. The results of these two-bottle tests suggest that an avoidance developed to the saccharin in the groups that were experiencing a bout of overeating at the time of saccharin availability.

It was mentioned previously that research into the nature of the decreased food consumption produced by injections of CCK produced competing theories as to whether this peptide was inducing satiety or a CTA. It was suggested by Cabanac and LaFrance (1990, 1991a, 1991b) that this dissociation may be too simplistic. They suggested that a component of satiety may in fact be aversive, and this may explain why studies were reporting evidence for both rewarding and aversive properties of CCK. The current experiments appear to support this suggestion. Using a natural behavioural paradigm involving self-induced overeating, a consistent avoidance of the novel solution that was paired with overeating was shown.

One issue that arose in this thesis was how the data from the experiments corresponds with the generally agreed upon definition of a CTA, in which rats choose to consume the non-associated flavour in a two-bottle preference test. The reduced saccharin consumption seen in the current experiments is a phenomenon that is relative, rather than absolute, in nature. During the acquisition trial(s) the overeating rats are consuming less saccharin than are the remaining groups, but are still consuming considerably more saccharin than water on a daily basis (data not reported). This result could be explained by the increased food availability, as rats that are consuming more food will generally consume more fluid as well (e.g. Kutscher, 1969). However, at the two-bottle test, when feeding has returned to normal, the overeating rats are still

consuming more saccharin than water on a daily basis. Further, at the two-bottle test, rats have access to both saccharin and water, and yet all groups consumed saccharin almost exclusively. These results suggest that while the overeating rats clearly found the saccharin less rewarding than did the rats that did not pair the saccharin with a period of overeating, they did not completely avoid it at the one-bottle or two-bottle tests.

This issue may be related to the nature of the design of the experiment. Because the rats were given 24 hr during which to consume saccharin in all the acquisition trials, even after the avoidance developed in the first trial, rats were forced to consume the solution again.

It is also noted that a complete avoidance of the conditioned flavour is not necessary to claim a CTA has developed. Studies using limited time access consumption tests have in fact claimed taste avoidances when consumption is decreased, as opposed to completely avoided (reviewed in Parker, 1995). Similarly, studies using 24 hr consumption tests have also claimed avoidances with only reduced consumption as opposed to complete avoidances (e.g. Parker & Gillies, 1995; Siegel, Parker, & Moroz, 1995). These studies have, however, generally reported the avoidance as a reduced preference compared to water.

It is theorized here that the apparently conflicting results seen in the current experiments (in that overeating rats will consume less saccharin than their relative controls, while still preferring saccharin to water in a two-bottle test) may be a result of the interaction of the rewarding and aversive aspects of satiation. As described previously, this is a constant matter of debate within the literature. Perhaps the rewarding aspects of feeding (such as the taste of the food and sweet solution) are conflicting with the negative aspects of consumption (the influx of calories and gastro-intestinal distension) resulting in a mix of positive and negative feedback.

It has been shown previously that stimuli, such as addicting drugs, that have demonstrated rewarding qualities in place preference and operant conditioning experiments, are

capable of producing CTAs (Reviewed in Parker, 1995, 2003). It was proposed that this avoidance is the result of conditioned "taste shyness" (Hunt & Amit, 1987). In this account, flavour avoidance is based on the alarm reaction to a novel internal stimulus, irregardless of whether the properties of this stimulus are positive or negative. It is suggested that any change in internal state in a rat produces this alarm reaction and this decreases consumption of any novel solution present.

While it is intuitive to suppose that the internal state change resulting from overeating is a negative one, the current experiments are not capable of determining the nature of the internal state change. It may be interesting in future studies to examine this phenomenon using a procedure that is thought to dissociate positive from negative changes in internal state.

The taste reactivity (TR) test (Grill and Norgren, 1978), involves the experimenter pairing the stimulus with a novel tasting solution; however, in this paradigm, the experimenter controls the amount of the flavour that the animal receives at test through an implanted catheter that infuses the flavour across the tongue. The experimenter measures the orofacial and somatic reactions that the animal demonstrates as a result of being exposed to the flavour. In this way it can be assessed whether the animal finds the flavour rewarding (it demonstrates consummatory responses such as tongue protrusions, mouth movements or paw licking) or aversive (it demonstrates rejection reactions such as gaping, chin rubbing or paw pushing). It has been shown previously that while conditioned taste avoidances can develop to both rewarding and aversive stimuli, disgust reactions in the TR test are not seen when this test is performed using stimuli that are rewarding (Parker, 1995). This would suggest that overeating might support a transition of TR from positive to one showing some aversive reactions or at least reduced consummatory responses.

In conclusion, the three experiments presented in this thesis demonstrated that overeating, when paired with the introduction of a novel solution, is capable of supporting a CTA using a 24 hr, ad lib access, experimental design. This finding was most evident in Experiment 2, where repeated pairings of the overeating and saccharin solution resulted in a long-lasting decrease in saccharin consumption. Experiment 3 then convincingly demonstrated that this avoidance was the result of a learned association as saccharin pre-exposure attenuated the avoidance in overeating rats.

References

Antin, J., Gibbs, J., Holt, J., Young, R. C., & Smith, G. P. (1975). Cholecystokinin elicits the complete behavioral sequence of satiety in rats. *Journal of Comparative and Physiological* Psychology, 89, 784-790.

Aoki, T., Grecu, T., Arcangeli, M. A., Benbarka, M. M., Prescott, P., & Ahn, J. H. (2001). Chronic intermittent intravenous insulin therapy: A new frontier in diabetes research. *Diabetes Technology & Therapeutics, 3*, 111-123.

Armstrong, S., Coleman, G., & Singer, G. (1980). Food and water deprivation: Changes in rat feeding, drinking, activity and body weight. *Neuroscience & Biobehavioral* Reviews, *4*, 377-402.

Babcock, A.M., Livosky, M. & Avery, D. D. (1985). Cholecystokinin and Bombesin suppress operant responding for food reward. *Pharmacology, Biochemistry, & Behavior, 22*, 893-895.

Bardos, G. (2001). Conditioned taste aversion to gut distension in rats. *Physiology & Behavior*, *74*, 407-413.

Berridge, K., Grill, H. J. & Norgren, R. (1981). Relation of consummatory responses and preabsorptive insulin release to palatability and learned taste aversions. *Journal of Comparative & Physiological Psychology, 95*, 363-382.

Best, M. R. (1975). Conditioned and latent inhibition in taste-avoidance learning: Clarifying the role of learned safety. *Journal of Experimental Psychology: Animal Behavior Processes*, *1*, 97-113.

Booth, D. A. (1985). Food-conditioned eating preferences and aversions with interoceptive elements: Conditioned appetites and satieties. *Annals of the New York Academy of Science, 443*, 22-41.

Bowers, R. L., Herzog, C. D., Stone, E. H. & Dionne, T. J. (1992). Defensive burying following injections of cholecystokinin, bombesin, and LiCl in rats. *Physiology and Behaviour, 51*, 969-972.

Cabanac, M & LaFrance, L. (1990). Postingestive alliesthesia: The rat tells the same story. *Physiology and behavior, 47*, 539-543.

Cabanac, M. & LaFrance, L. (1991a). Facial consummatory responses in rats supports the ponderostat hypothesis. *Physiology and Behavior, 50*, 179-183.

Cabanac, M. & LaFrance, L. (1991b). Ingestive/Aversive responses of rats to sweet stimuli. Influence of glucose, oil, and casein hydrolyzate gastric loads. *Physiology and Behavior, 51*, 139-143.

Campfield, L. A., Smith, F. J., Rosenbaum, M., & Hirsch, J. (1996). Human eating: Evidence for a physiological basis using a modified paradigm. *Neuroscience & Biobehavioral Reviews, 20*, 133-137.

Collier, G. H. (1986). The dialogue between the house economist and the resident physiologist. *Nutrition & Behaviour, 3*, 9-26.

Collier, G. H. (1989). The economics of hunger, thirst, satiety, and regulation. *Annals of the New York Academy of Sciences, 575*, 136-154.

Collier, G. H. & Johnson, D. F. (1990). The time window of feeding, *Physiology & Behavior, 48*, 771-778.

Collier, G. H., Johnson, D. F., Hill, W. L., & Kaufman, L. W. (1986). The economics of the law of effect. *Journal of the Experimental Analysis of Behaviour, 48*, 113-136.

Collier, G. H., Johnson, D. F., & Morgan, C. (1992). The magnitude-of-reinforcement function in closed and open economics. *Journal of the Experimental Analysis of Behavior, 57*, 81-89.

Deutsch, R. (1974). Conditioned hypoglycemia: A mechanism for saccharin-induced sensitivity in the rat. *Journal of Comparative and Physiological Psychology, 86*, 350-358.

Deutsch, J. A. & Hardy, W. T. (1977). Cholecysotokinin produces bait shyness in rats. *Nature, 266*, 196.

Drucker, D. B., Ackroff, K., Sclafani, A. (1994). Nutrient-conditioned flavour preference and acceptance in rats: Effects of deprivation state and nonreinforcement. *Physiology & Behavior, 56*, 701-707.

Elizalde, G. & Sclafani, A. (1990). Flavor preferences conditioned by intragastric polycose infusions: A detailed analysis using an electronic esophagus preparation. *Physiology & Behavior, 47*, 63-77.

Evans, S. A., Messina, M. M., Knight, W. D., Parsons, A. D. & Overton, J. M. (2005). Long-Evans and Sprague-Dawley rats exhibit divergent responses to refeeding after caloric restriction. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 288*, R1468-R1476.

Even, P. & Nicolaidis, S. (1985). Spontaneous and 2DG-induced metabolic changes and feeding: The ischymetric hypothesis. *Brain Research Bulletin, 15*, 429-435.

Forristall, J. R., Hookey, B. L., & Grant, V. L. (2007). Conditioned taste avoidance induced by forced and voluntary wheel running in rats. *Behavioral Processes, 74*, 326-333.

Gamzu, E. (1977). The multifaceted nature of taste-aversion inducing agents: Is there a single common factor? In: Barker, L.; Best, M.; 30. Domjan, M. eds. Learning mechanisms in food selection. Waco, Texas: Baylor University Press.

Gibbs, J., Young, R. C. & Smith, G. P. (1973). Cholecystokinin decreases food intake in rats. *Journal of Comparative and Physiological Psychology, 84*, 488-495.

Gray, D. S., Fisler, J. A., & Bray, G. A. (1988). Effects of repeated weight loss and regain on body composition in obese rats. *American Journal of Clinical Nutrition, 47*, 393-399.

Grill, H. J. & Norgren, R. (1978). The taste reactivity test: I. Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain Research, 143*, 263-279.

Harris, R. B. S. & Martin, R. J. (1984). Recovery of body weight from below weight "set point" in mature female rats. *Journal of Nutrition, 114*, 114301150.

Havel, P. J. (2000). Role of adipose tissue in body-weight regulation: Mechanisms regulating leptin production and energy balance. *Proceedings of the Nutrition Society, 59*, 359-371.

Havel, P.J. (2001). Peripheral signals conveying metabolic information to the brain: Short-term and long-term regulation of food intake and energy homeostasis. *Experimental Biology and Medicine, 226*, 963-977.

Hertel, A., Botzang, L., Parfeniuk, G. G., & Eikelboom, R. (In Press). The effects of initial weight on the wheel-induced feeding suppression in rats. *Behavioral Processes.*

Heth, D. C., & Pierce, D. W. (2007). The role of pre-exposure to novel food tastes in activity-based conditioned taste avoidance. *Learning and Motivation, 38*, 35-43.

Hunt, T. & Amit, Z. (1987). Conditioned taste aversion induced by self-administered drugs: Paradox revisited. *Neuroscience & Biobehavioral Reviews, 11*, 107-130.

Hursh, S. R. & Beck, R. C. (1971). Bitter and sweet saccharin preferences as a function of food deprivation. *Psychological Reports, 29*, 419-422.

Iverson, I. H. (1993). Techniques for establishing schedules with wheel running as reinforcement in rats. *Journal of the Experimental Analysis of Behaviour, 60*, 219-238.

Konorski, J. (1967). Integrative activity of the brain: An interdisciplinary approach. Chicago: University of Chicago Press.

Kutscher, C.L. (1969). Species differences in the interaction of feeding and drinking. *Annals of the New York Academy of Sciences, 159*, 539-551.

Lett, B. T., & Grant, V. L. (1996). Wheel running induces conditioned taste aversion in rats trained while hungry and thirsty. *Physiology & Behavior, 59*, 699-702.

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

Lett, B. T. Grant, V. L., Koh, M. T., & Smith, J. F. (2001). Wheel running simultaneously produces conditioned taste aversion and conditioned place preference in rats. *Learning & Motivation, 32*, 129-136.

Lockard, R. B. (1967). Replacement behavior following deprivation of food, water, running, or light. *Psychological Reports, 21*, 753-769.

Lubow, R. E. (1973). Latent inhibition. *Psychological Bulletin, 79*, 398-407.

Lubow, R. E., & Moore, A. U. (1959). Latent Inhibition: The effect of nonreinforced pre-exposure to the conditional stimulus. *Journal of Comparative and Physiological Psychology, 52*, 415-419.

Maddison, S. (1977). Intraperitoneal and intracranial cholecystokinin depress operant responding for food. *Physiology & Behavior, 19*, 819-824.

McCutcheon, B., Ballard, M. & McCaffrey, R. J. (1992). Intraperitoneally injected cholecystokinin-octapeptide activates pica in rats. *Physiology & Behavior, 51*,543-547.

Mitchell, D., Wells, C., Hoch, N., Lind, K., Woods, S. C. & Mitchell, L. K. (1976). Poison induced pica in rats. *Physiology and Behavior, 17*, 691-697.

Moran, T. H. (2000). Cholecystokinin and satiety: Current perspectives. *Nutrition, 16,* 858-865.

Myers, K. P., Ferris, J., & Sclafani, A. (2005). Flavor preferences conditioned by postingestive effects of nutrients in preweanling rats. *Physiology & Behavior, 84,* 407-419.

Nakajima, S., & Nagaishi, T. (2005). Summation of latent inhibition and overshadowing in a generalized bait shyness of rats. *Behavioral Processes, 69,* 369-377.

Parker, L. A. (1982). Nonconsummatory and consummatory behavioural CRs elicited by lithium- and amphetamine-paired flavours. *Learning & Motivation, 13,* 281-303.

Parker, L. A. (1995). Rewarding drugs produce taste avoidance but not taste aversion. *Neuroscience and Biobehavioral Reviews, 19,* 143-151.

Parker, L .A. (2003). Taste avoidance and taste aversion: Evidence for two different processes. *Learning & Behavior, 31,* 165-172.

Parker, L. A. & Gillies, T. (1995). THC-induced place and taste aversions in lewis and Sprague-dawley rats. *Behavioral Neuroscience, 109,* 71-78.

Picard, F., Naimi, N., Richard, D., & Deshaies, Y. (1999). Response of adipose tissue lipoprotein to the cephalic phase of insulin secretion. *Diabetes, 48,* 452-459.

Quimby, F. H. (1948). Food and water economy of the young rat during chronic starvation and recovery. *Journal of Nutrition, 36,* 177-186.

Reicher, M. A. & Holman, E. W. (1977). Location preference and flavour aversion reinforced by amphetamine in rats. *Animal Learning & Behavior, 5,* 343-346.

Revusky, S. H. (1968). Aversion to sucrose produced by contingent X-irradiation: Temporal and dosage parameters. *Journal of Comparative and Physiological Psychology, 65,* 17-22.

Robertson, M. D., Jackson, K. G., Williams, C. M., Fielding, B. A., & Frayn, K. N. (2001). Prolonged effects of modified sham feeding on energy substrate mobilization. *American Journal of Clinical Nutrition, 73*, 111-117.

Rushing, P. A. & Gibbs, J. (1998). Prolongation of intermeal interval by gastrin-releasing peptide 1-27 depends on time of delivery. *Peptides, 19*, 1439-1442.

Rushing, P .A., Henderson, R .P. & Gibbs, J. (1998). Prolongation of the postprandial intermeal interval by gastrin-releasing peptide 1-27in spontaneously feeding rats. *Peptides, 19*, 175-177.

Satvat, E. & Eikelboom, R. (2006). Dissociation of conditioned and unconditioned factors in the running-induced feeding suppression. *Physiology & Behavior, 89*, 428-437.

Schwartz, M. W., Woods., S. C., Porte, D. J., Seeley, R. J., & Baskin, D. G. (2000). Central nervous system control of food intake. *Nature, 404*, 661-671.

Sclafani, A., Nissenbaum, J. W., & Ackroff, K. (1994). Learned preferences for real-fed and sham-fed polycose in rats. Interaction of taste, postingestive reinforcement and satiety. *Physiology & Behavior, 56*, 331-337.

Shillabeer, G. & Davison, J. S. (1982). The cholecystokinin antagonist, proglumide, increases food intake in the rat. *Regulatory Peptides, 8*, 171-176.

Shillabeer, G. & Davison, J. S. (1985). Increased food intake in the rat caused by proglumide, the cholecystokinin antagonist: An effect abolished by vagotomy. *Annuals of the New York Academy of Science, 448*, 648-650.

Siegel, S., Parker, L. A., & Moroz, I. (1995). Morphine-induced taste avoidance is attenuated with multiple conditioning trials. *Pharmocology, Biochemistry, and Behavior, 50*, 299-303.

Smith, G. P. (Ed.) (1998). *Satiation: From Gut to Brain.* New York: Oxford University Press.

Smith, G. P. & Gibbs, J. (1994). Satiating effects of Cholecystokinin. *Annuals of the New York Academy of Science, 713*, 236-241

Smith, J. C. & Roll, D. L. (1967). Trace conditioning with X-rays as an aversive stimulus. *Psychonomic Science, 9,* 11-12.

Tordoff, M. G., Alarcon, L. K., & Lawler, M. P. (2008). Preferences of 14 rat strains for 17 taste compounds. *Physiology & Behavior, 95*, 308-332.

Valenstein, E. S. (1967). Selection of nutritive and non-nutritive solutions under different conditions of need. *Journal of Comparative and Physiological Psychology, 63*, 429-433.

Van Vort, ,W. & Smith, G. P. (1983). The relationship between the positive reinforcing and satiating effects of a meal in the rat. *Physiology & Behavior, 30*, 279-284.

Van Vort, W. & Smith, G. P. (1987). Sham feeding produces a conditioned increase in meal size. *Appetite, 9*, 21-29.

Warwick, Z. S. & Weingarten, H. P. (1996). Flavor-postingestive consequence associations incorporate the behaviorally-opposing effects of positive reinforcement and anticipated satiety: Implications for interpreting two-bottle tests. *Physiology and Behavior, 60*, 711-715.

Wise, R., Yokel, P., & Dewitt, H. (1976). Both positive reinforcement and conditioned aversion from amphetamine and apomorphine in rats. *Science, 191*, 1273-1274.

Wolgin, D. L. & Wade, J. V. (1990). Effect of lithium chloride-induced aversion on appetitive and consummatory behavior. *Behavioral Neuroscience, 104*, 438-440.

Woods, S. C. (1991). The eating paradox: How we tolerate food. *Psychological Review, 98*, 488-505.

Woods, S. C., Seeley, R. J., Porte, D. J., & Schwartz, M. W. (1998). Signals that regulate food intake and energy homeostasis. *Science, 280,* 1378-1383.

Woods, S. C. & Strubbe, J. H. (1994). The psychobiology of meals. *Psychonomic Bulletin & Review, 1,* 141-155.

Figure Captions

Figure 1

Procedural time line for Experiment 1.

Figure 2

Procedural time line for Experiment 2.

Figure 3

Procedural time line for Experiment 3.

Figure 4

Mean (+SEM) grams of food consumed in Experiment 1. During the restriction phase, restricted rats were consuming a fixed amount of food each day. At the acquisition trial, the rest-adlib group was consuming significantly more food than the remaining groups. At the time of the two bottle test, all rats were consuming approximately the same amount of food.

Figure 5

Mean (+SEM) body mass of rats in Experiment 1. Food restriction reduced body mass gain in rats compared to the adlib groups. Access to adlib (and to a lesser extent 100%) food at the saccharin acquisition day resulted in body mass gain. Mass gain in the adlib-adlib group was consistent throughout the experiment.

Figure 6

Mean (+SEM) grams of saccharin consumed at the acquisition trial in Experiment 1. Rats experiencing a change in feeding schedule at this time (rest-100% and rest-adlib) consumed significantly less saccharin than did those groups not experiencing a change in feeding (rest-rest and adlib-adlib).

Figure 7

Mean (+SEM) grams of saccharin and water consumed at the two-bottle test in Experiment 1. There were no longer any significant group differences in consumption. Pre-planned comparisons revealed a significant difference between the adlib-adlib and rest-adlib groups. Water consumption was very low for all groups.

Figure 8

Mean (+SEM) grams of food consumed in Experiment 2. During the restriction phase, restricted rats were consuming a fixed amount of food each day. At all the acquisition trials, the rest-adlib group was consuming significantly more food than the remaining groups. At the time of the two bottle test, all rats were consuming approximately the same amount of food.

Figure 9

Mean (+SEM) body mass of rats in Experiment 2. Food restriction reduced body mass gain in rats compared to the adlib groups. Access to adlib (and to a lesser extent 100%) food at the saccharin acquisition days resulted in mass gain. Mass gain in the adlib-adlib group was consistent throughout the experiment.

Figure 10

Mean (+SEM) grams of saccharin consumed over the three saccharin acquisition trials in Experiment 2. Rats that experienced a change in food consumption (rest-100% and rest-adlib) were consuming significantly less saccharin than were groups that were not experiencing a change in food consumption (rest-rest and adlib-adlib). Further the rest-adlib group was consuming significantly less saccharin than the rest-100% group.

Figure 11

Mean (+SEM) grams of saccharin and water consumed at the two-bottle test in Experiment 2. The rest-adlib group was consuming significantly less saccharin than were the

remaining groups. The rest-100% group was no longer consuming less saccharin than the adlib-adlib and rest-rest groups. Water consumption was very low for all groups.

Figure 12

Mean (+SEM) grams of food consumed in Experiment 3. Saccharin pre-exposure had no effect on food consumption. During the restriction phase the restricted groups were consuming a fixed amount of food. At each acquisition trial the restricted rats were consuming significantly more food than were the adlib groups. The adlib groups were approximately equal in food consumption throughout the experiment. At the time of the two-bottle test, food consumption in all groups was approximately equal.

Figure 13

Mean (+SEM) body mass of rats in Experiment 3. Saccharin pre-exposure had no effect and food restriction significantly reduced mass gain. Adlib food consumption in previously restricted rats on the three saccharin acquisition days resulted in increased mass gain. Body mass gain was approximately equal in the two adlib and two restricted groups.

Figure 14

Mean (+SEM) grams of saccharin consumed during the saccharin pre-exposure phase in Experiment 3. Both the future restricted and future adlib groups were consuming approximately equal amounts of saccharin.

Figure 15

Mean (+SEM) grams of saccharin consumed over the three acquisition trials in Experiment 3. The rest-rest group was consuming significantly less saccharin than were the remaining groups. There was a near significant Exposure x Restriction interaction on saccharin consumption ($p=0.07$) No other group differences were significant.

Figure 16

Mean (+SEM) grams of saccharin and water consumed at the two-bottle test in Experiment 3. The rest-rest group was consuming significantly less saccharin than were the remaining groups. There was a significant Exposure x Restriction interaction on saccharin consumption ($P<0.05$). Water consumption was very low for all groups.

Figure 1: Experiment 1

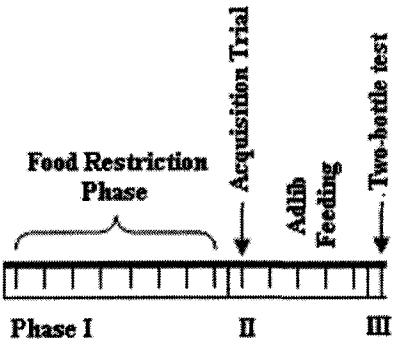

Figure 2: Experiment 2

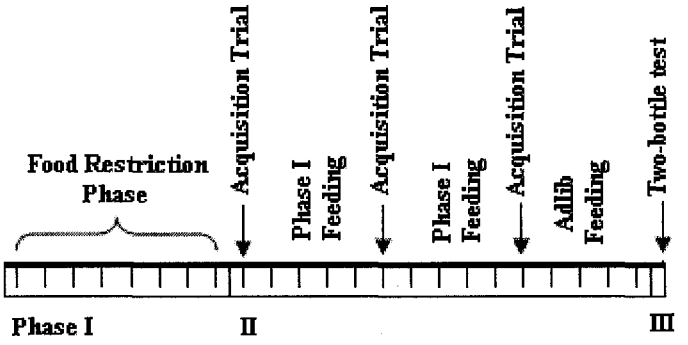

Figure 3: Experiment 3

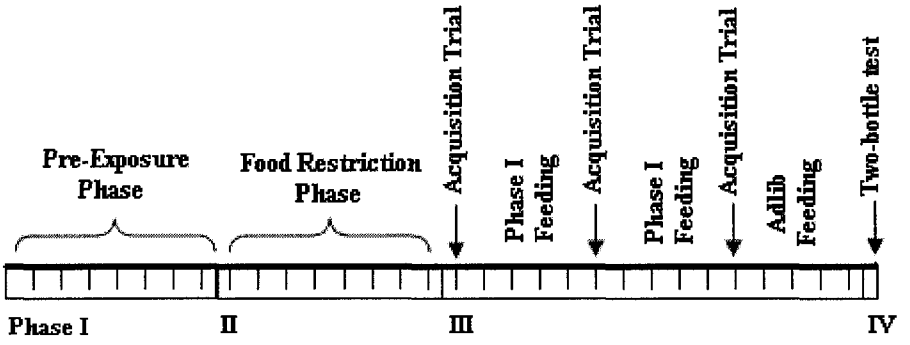

Figure 4: Food Consumption

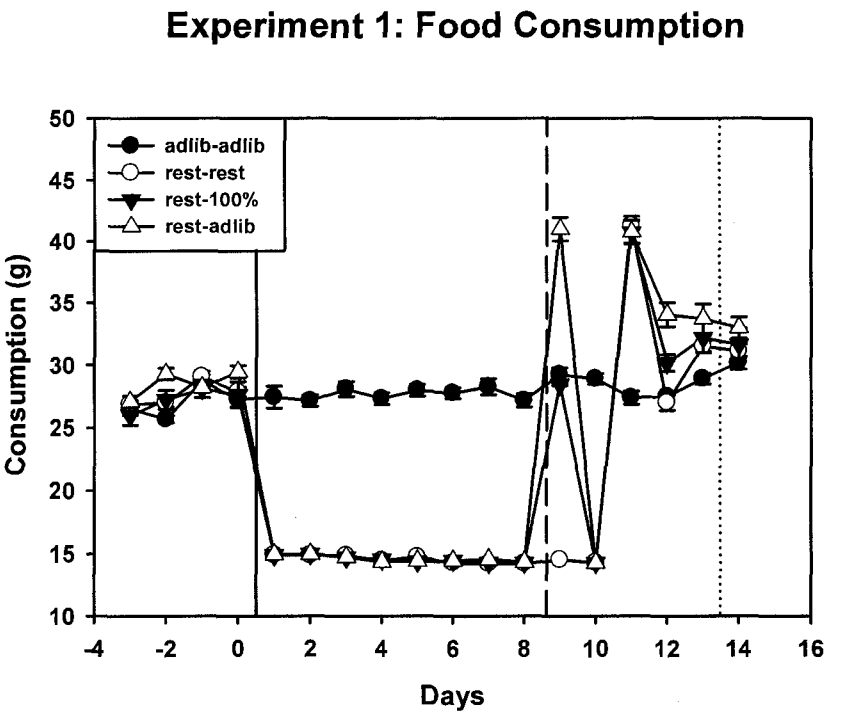

Figure 5: Body Mass

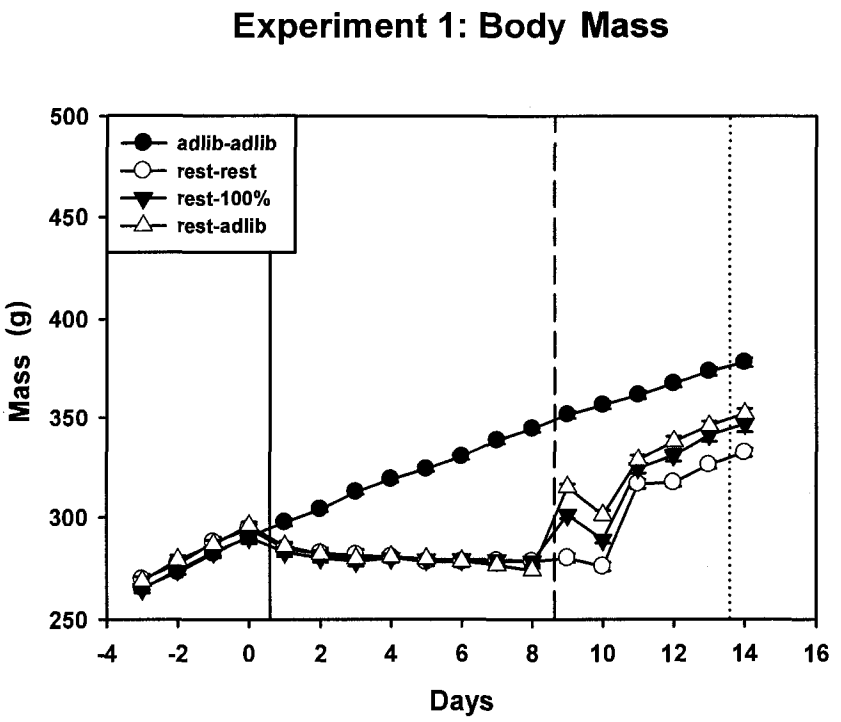

Figure 6: Saccharin Consumption (Acquisition Trial)

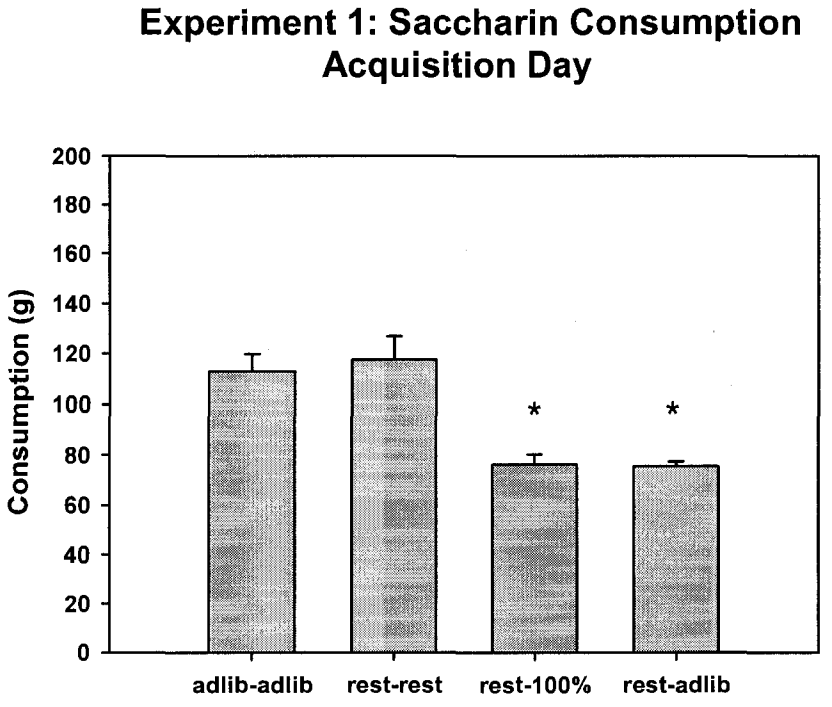

Figure 7: Saccharin and water consumption (two-bottle test)

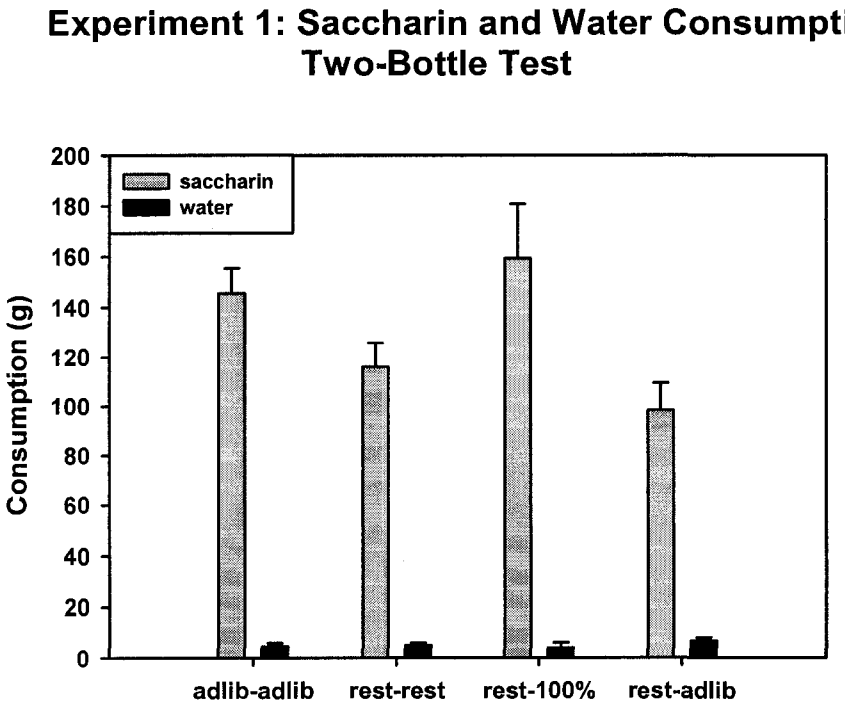

Figure 8: Food Consumption

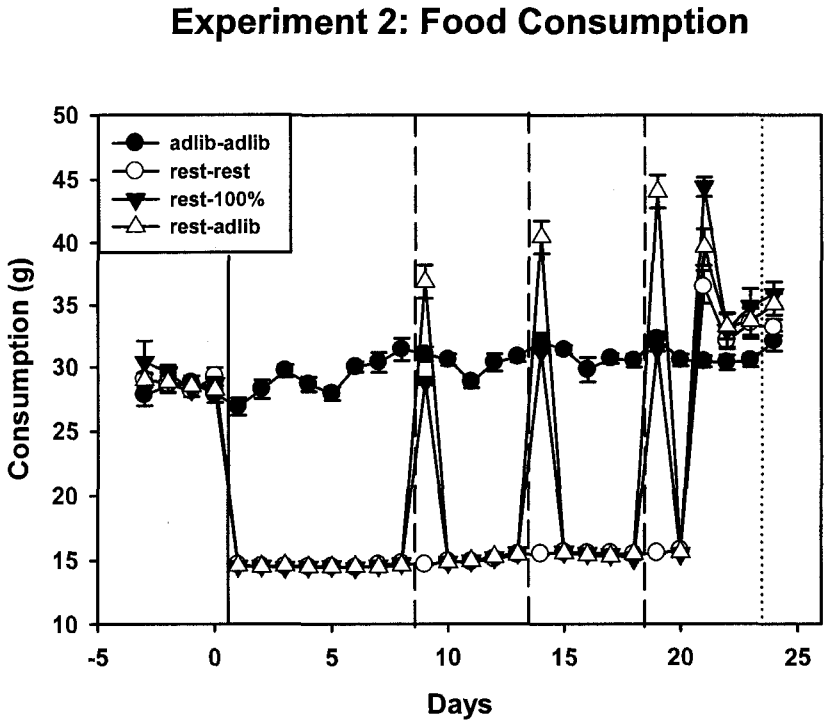

Figure 9: Body Mass

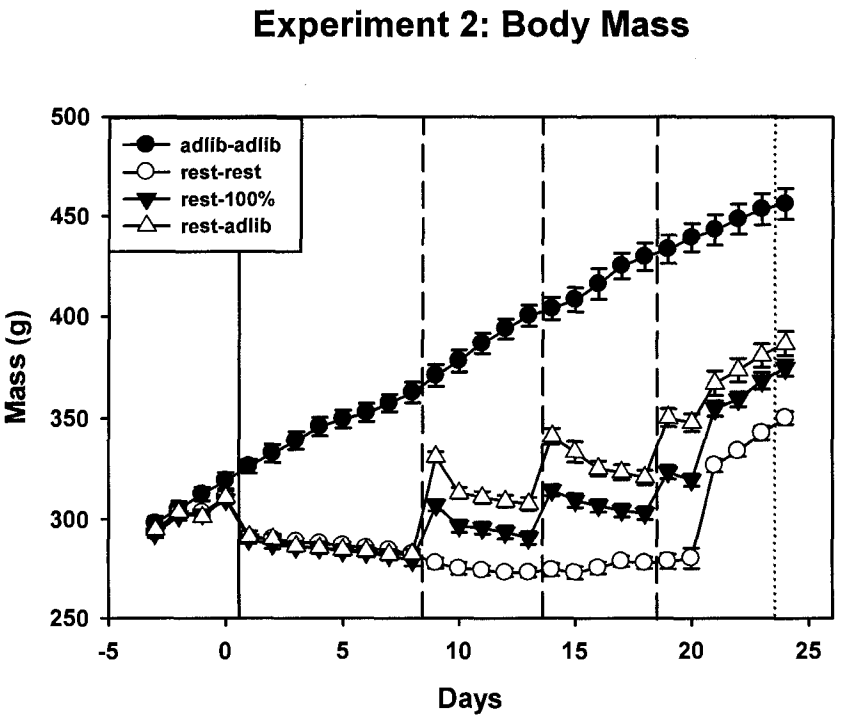

Figure 10: Saccharin consumption (Acquisition trials)

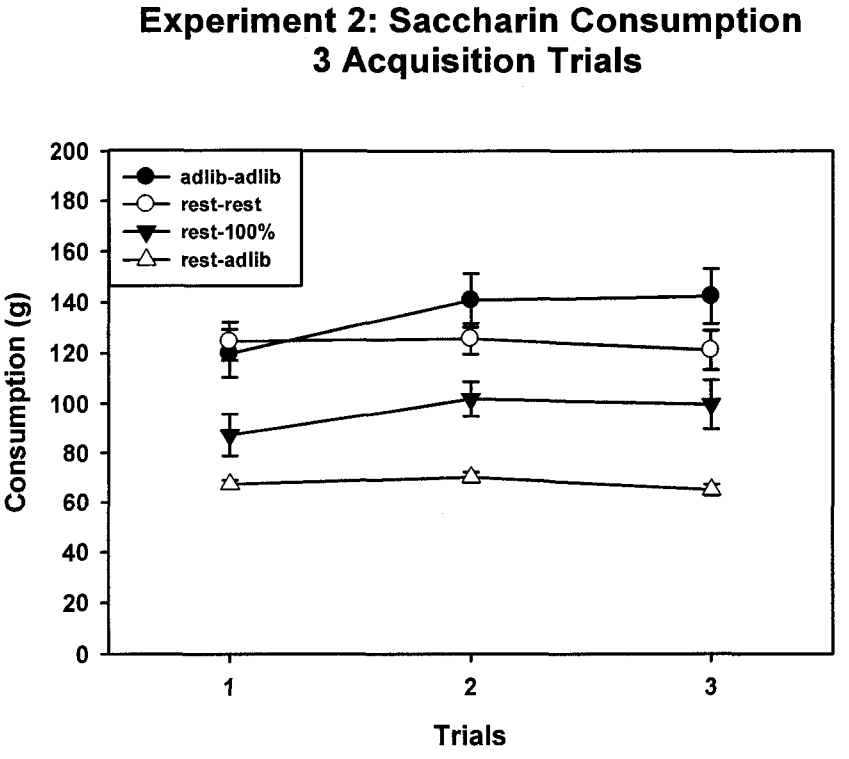

Figure 11: Saccharin and water consumption (two-bottle test)

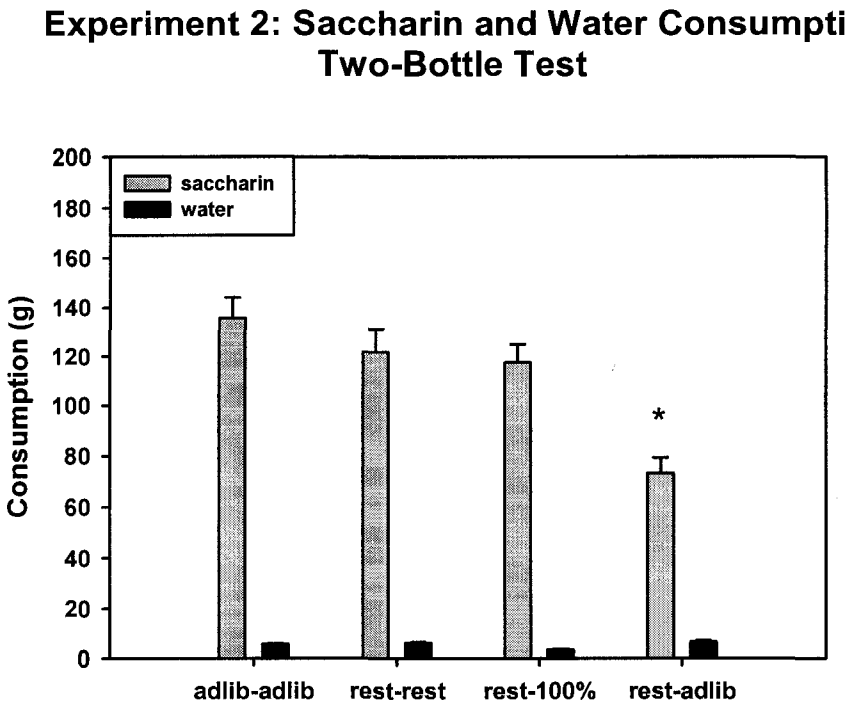

Figure 12: Food Consumption

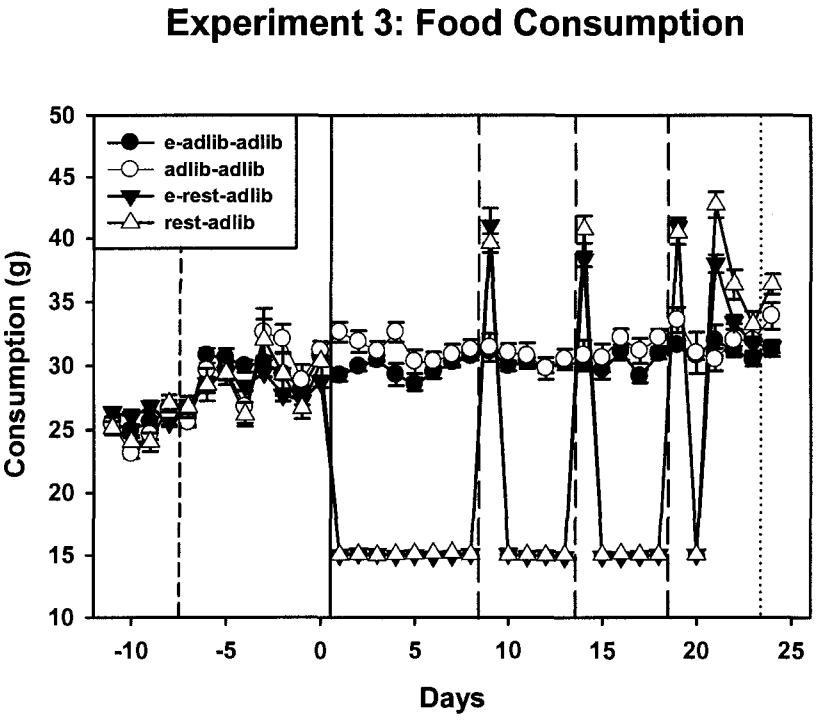

Figure 13: Body Mass

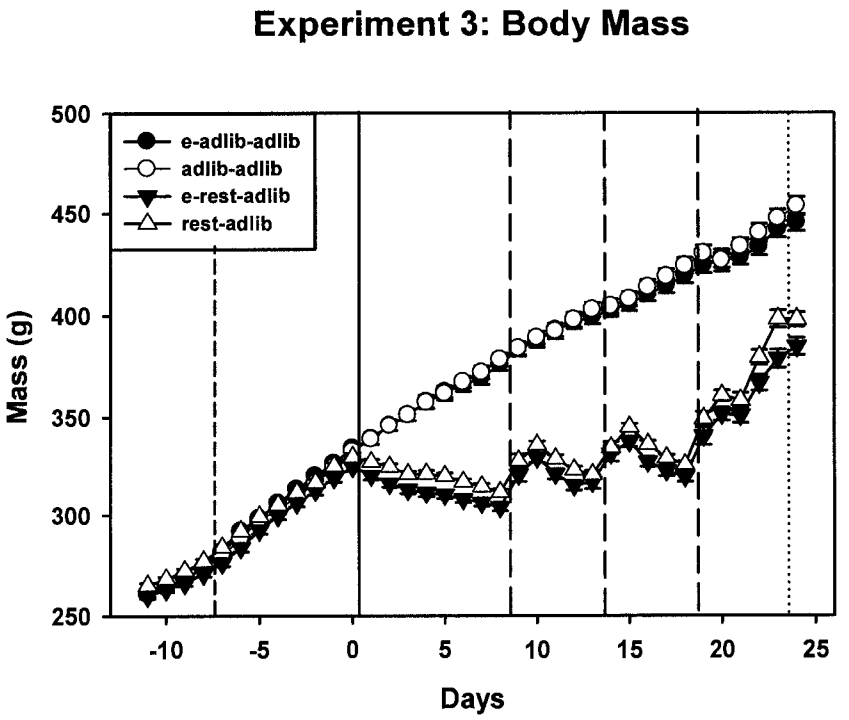

Figure 14: Saccharin Consumption (pre-exposure)

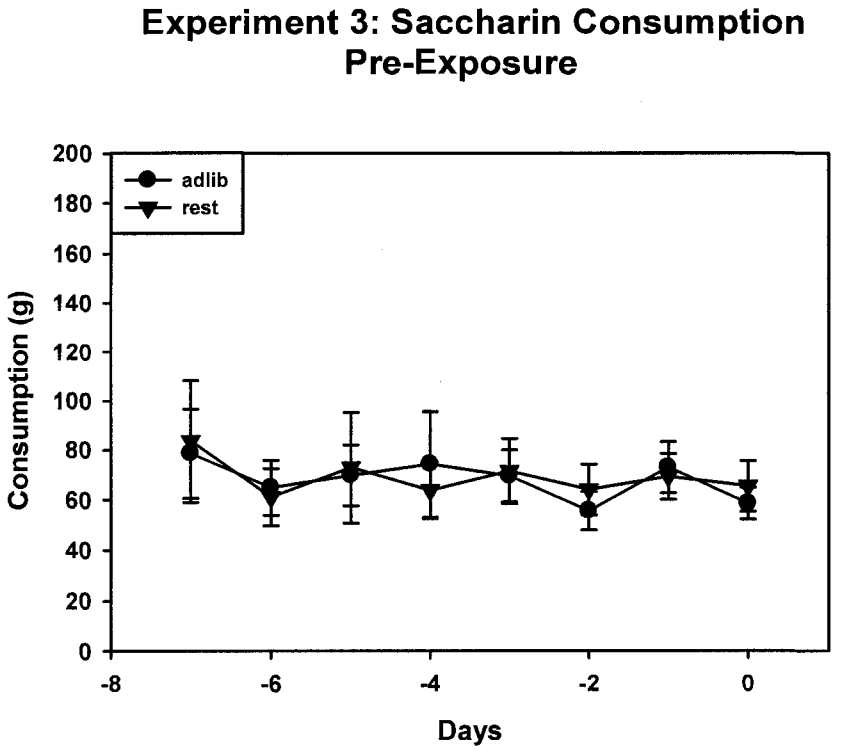

Figure 15: Saccharin Consumption (acquisition trials)

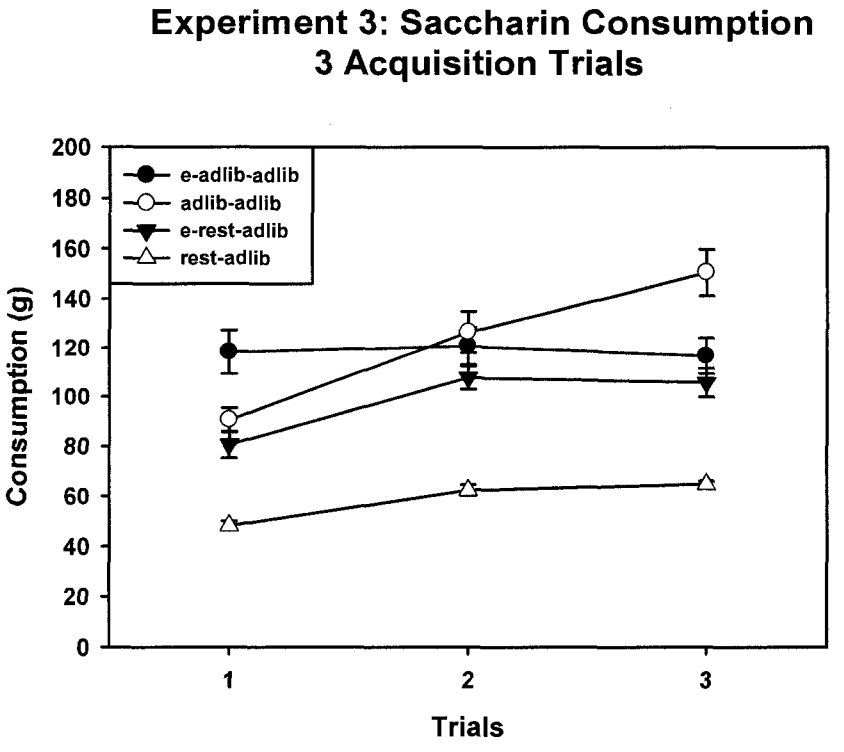

Figure 16: Saccharin and Water Consumption (Two-Bottle Test)