The Effects of Stress on Food Preferences of High-Saccharin and Low-Saccharin Preferring Rats

by

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Abstract

High- Saccharin (HiS) and Low- Saccharin (LoS) preferring strains of rats are found to differ in preference for a saccharin solution, as well as reactivity to stress, drug seeking behaviour, and taste preferences. Because of this unique intersection of characteristics these rat strains were ideal for use in this study which examined how stress influences the consumptive behaviour of the HiS versus the LoS lines for flavoured foods instead of flavoured liquids (as used in the previous literature). Predictions were made within a sensation seeking/optimal arousal framework (SS/OA). The fourteen HiS (*n*= 7) and LoS (*n*= 7) rats received a restraint stressor for 30 minutes. Their consumptive behaviours were then monitored for 30 minutes. The stressor significantly reduced the consumption of the two lines. However, the taste preferences of the animals were not found to support SS/OA theory. It was posited that the addition of calories to the test foods of the current study influenced the consumption and taste preferences of the HiS and LoS lines in a way that is not predicted by the sensation seeking/ optimal arousal theory.

Keywords: stress, eating, taste preferences, high saccharin preference, low saccharin preference, caloric foods, non-caloric foods

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1 Introduction

Within the current literature on stress and its effects on consumption, there are contradictory results, citing both an increase and a decrease in consumption during stress. This finding has been observed in both human and rat populations (Hagan et al., 2003; Howell et al., 1999; Krebs et al., 1996; Mitchell & Epstein, 1996). Many possible explanations for this contradiction have been cited ranging from types of stress being applied to what food is made available (Krebs, Macht, Weyers, Weijers, & Janke, 1996; Macht, Krebs, Weyers &, Janke, 2001; Teegarden & Bale, 2008). Stress has also been shown to affect food preferences as well as the amount of food consumed (Howell et al., 1999; Tarjan & Denton, 1991). To help clarify possible genetic influences on the effects of stress on ingestive behaviour, different rat strains have been used to help identify what role genetics play in eating during stress (Dess & Minor, 1996; Tordoff, Alarcon, & Lawler, 2008). For example, the high-saccharin-preferring (HiS) and low-saccharin-preferring (LoS) strains have been bred to differ on saccharin preference in a two-bottle preference test; HiS rats exhibit greater preference scores for saccharin than the LoS rats with the preference scores of the LoS rats trending towards a greater preference for water. While being bred to differ on saccharin preference, the HiS and LoS rats have also been found to differ in their taste preferences for flavours other than saccharin (Dess, 2000). Along with their characteristic taste preferences, the HiS and LoS strains were also found to differ in emotionality (Dess & Minor, 1996). This discovery made the HiS and LoS strain popular in studying the relationship between emotionality and consumption, as they will be used in the current study. These strains were chosen for the current study for their different sets of heritable traits, specifically their differing taste preferences. The intention of this study was to identify the food preferences of the HiS and LoS strains and to then determine if stress affects these food

preferences. The existing literatures concerning food preferences and the effects of stress on consumption and food preference are vast; however, literature does not yet exist concerning the effects of stress on the food preferences of the HiS and LoS rat strains. Thus the current study was executed with the intention of exploring this gap in the literature. This particular line of study is important because it will help to illuminate how the effect of stress on food consumption varies with different innate taste preferences.

The purpose of this study is to build on previous findings and gain a further understanding of how breeding rats for certain taste preferences (i.e. ones that highly prefer saccharin and ones that do not) also affects their preferences for other tastes. The specific goal of this study is to see if these heritable taste preferences can also be influenced by a stressor. In rats not bred for taste preferences, stress can produce a stronger preference for sweet foods, so the question this study hopes to answer is if this stress produced preference is magnified in rats bred to prefer sweet tastes (i.e. saccharin) or minimized in rats bred not to prefer sweet tastes.

To accomplish this goal within the present study, two strains of rats with different heritable traits (the HiS and the LoS rats) were subjected to a restraint stressor. Following this stressor the rats were presented with different flavoured foods, and their consumption of these foods was observed and compared to one another.

A secondary goal of the present study was to recreate the taste preference results of the previous literature but within a more ecologically valid setting that better represents normal consumptive behaviours (Dess, 2000). This was accomplished by using flavoured foods to test taste preferences and consumption during stress instead of using flavoured liquids like the previous literature (Dess, 2000). The use of solid food is more ecologically valid because both

rats and humans gain most of their nourishment and calories through the consumption of foods rather than liquids.

The applications/ implications of this research are foundational in nature. Meaning that although this study does not directly test the effects of stress on heritable traits in humans, the results from the current study can be used to make predictions regarding humans which can then inform and become the foundation for future research.

1.1 HiS and LoS rat research

The HiS and LoS strains being used in this study were originally bred by Nachman (1959). Through selective breeding of the highest and lowest saccharin preferring rats (highs mated with other highs and lows mated with other lows), he managed to produce two strains of rats; one strain had a strong preference for a liquid saccharin solution over water and the other had a greater preference for water over the liquid saccharin solution. This supported the idea that saccharin preference and perhaps sweet taste preference is influenced by heritable traits. From those beginnings they have been used in many other research applications ranging from drug abuse studies to anxiety studies (Carroll, Anderson & Morgan, 2007; Carroll et al., 2008; Dess & Minor, 1996).

The HiS and LoS rats are strains that have been bred to have greater or lesser preference scores for a sodium saccharin (referred to as saccharin) solution (0.10%) in a two-bottle preference test. The research regarding the HiS and LoS strains has found that these strains differ not only in saccharin consumption but also with regard to propensity for drug abuse, impulsivity, reactivity to stress, taste preference, and consumption during stress (Carroll, Anderson & Morgan, 2007; Caroll et al., 2008; Dess & Minor, 1996; Dess, 2000; VanderWeele, Dess &

Castonguay, 2002). The intersection of these unique characteristics makes the HiS and LoS rats ideal for use in studies regarding stress and eating, which is why they were chosen for the current study. Findings from the present study add to the existing body of literature regarding the eating habits and food preferences of these strains when under stress. Studies regarding consumption during stress in general are always beneficial, as the complex relationship between these two variables has yet to be fully understood. For example, everyone experiences some degree of stress in their daily lives, and additionally obesity is a health risk that is increasing in prevalence. Thus it is important to understand how stress influences consumption so that changes in consumption can be predicted, and controlled for so that people may maintain a healthy body weight.

The literature concerning the taste preferences of the HiS and LoS strains as well as their food consumption during stress indicates that these strains differ on various taste preferences as well as on the amount of food consumed in response to a stressor (Dess & Minor, 1996; Dess, 2000). A study by Dess (2000) provides evidence suggesting that LoS rats and HiS rats have different taste preferences for various flavoured liquid solutions. As compared to the HiS rats, LoS rats have a weaker preference/lower consumption of sweet, salty and starchy liquid solutions. The strains, however, did not differ in their consumption of bitter or sour liquid solutions. Additionally, there is evidence that the HiS and LoS rats also differ in their eating response to a stressor. A study by Dess and Minor (1996), revealed that, in response to a shock stressor, the LoS rats reduced consumption of their regular rat chow while the consumption of the HiS rats remained the same. This finding suggests that stress results in decreased food consumption; however this effect appears to be dependent upon genetics. Due to the differing

genetic makeup of the HiS and LoS rats, decreased consumption in response to the stressor was only seen with the LoS rats and not with the HiS rats.

Although the consumption habits of the HiS and LoS strains appear to have been investigated thoroughly, there has yet to be any literature regarding the effects of stress on the food preferences of the HiS and LoS strains, as well as the food preferences of these strains for flavoured solids (as opposed to flavoured liquids). Since animals typically ingest their calories through the consumption of solid foods (rather than caloric liquids) this aspect of the study seeks to provide a more ecologically sound basis to the results than previous studies that used liquids. The current study was performed with the intention of investigating these aspects, and thus serves an important function in the literature by addressing these gaps. Addressing these gaps will help to inform how different innate taste preferences and how the consumption of different flavoured foods influences how stress affects consumptive behaviours. Specifically the current study involves the use of flavoured foods containing calories, while all the previous studies used flavoured liquids that contained no calories. It is possible that these two different conditions, solids containing calories versus liquids containing no calories, will elicit different consumptive behaviours from the animals. Using solid food examines the possibility that some post-oral effect of the consumed calories/food has an effect on the food preferences of the rats. This is a phenomenon called alliesthesia and has been cited in the literature, which will be reviewed later on (Berridge, 1991).

1.2 HiS and LoS rats and the Sensation Seeking Paradigm

The different characteristics of the HiS and LoS strains (drug-seeking behaviour, emotionality, etc.) seem to draw a parallel with the characteristics of people who are and are not

sensation seekers (Blanchard, Mendelsohn & Stamp, 2009; Carroll et al., 2002; Carroll, Anderson & Morgan, 2007; Carroll et al., 2008; Dess & Minor, 1996; Dess, 2000; Pliner& Melo, 1997). This suggests that Nachman, Dess and Carroll may have unintentionally selected for sensation seekers versus non-sensation seekers, while selecting for high and low saccharin preferring rats. Sensation seekers in the literature have been found to have a higher pain tolerance, participate in risk taking, exhibit a propensity to seek out new stimuli and are more vulnerable to drug abuse (Blanchard, Mendelsohn & Stamp, 2009; De Pascalis Valerio, Santoro, & Cacace, 2007). The mechanism driving the sensation seeking model is the concept of optimal arousal. This concept states that within the human population there are people who differ on the level of arousal at which they are most comfortable, and those who differ on their baseline arousal (Pliner & Melo, 1997). Thus individuals would be motivated to sensation seek if their arousal level is below optimal, or be motivated to avoid extra stimulation if their arousal level is above or already at optimal (Pliner& Melo, 1997). In line with the optimal arousal mechanism, sensation seekers are individuals who are less easily stimulated and have a higher threshold for optimal arousal. This makes them more likely to look for ways to increase their arousal levels. This is unlike the non-sensation seekers who are individuals that have a lower threshold for optimal arousal and are more easily stimulated.

Because of the parallels in behaviour observed between the sensation seekers and non-seekers and the HiS and LoS strains, the sensation seeking model was used to frame the hypotheses of the current study. The HiS rats are the sensation seekers and the LoS rats are the non-sensation seekers. Within this framework the LoS rats are highly aroused all the time and thus any small amount of stimulation can be unpleasant, such as a sweet food or a drug. Stimulation would be unpleasant as a small increase would result in a deviation from optimal

arousal; the HiS rats are not as easily aroused and thus are more prone to enjoy highly stimulating things such as sweet foods and drugs. Stimulation of the HiS rats would move their arousal level closer to optimal, and thus stimulation would be pleasant. It is even possible that the HiS strain is chronically under aroused resulting in a propensity to sensation seek just to maintain an optimal level of arousal. This is supported by a study in which the HiS rats were found to have a lower baseline arousal level than the LoS rats (VanderWeele, Dess & Castonguay, 2002). The current study examined the possibility that the sensation seeking model could explain some of the behavioural differences between the HiS and LoS strains.

The sensation seeking model appears to neatly summarize and explain the differences among the HiS and LoS strains. For example, both the HiS rats and sensation seekers show a propensity for drug administration, a higher pain tolerance, a propensity for taking risks, and a propensity to seek out new stimuli when compared to their LoS strain or non-sensation seeking counterparts (Blanchard, Mendelsohn & Stamp, 2009; Carroll et al., 2002; Carroll, Anderson & Morgan, 2007; Carroll et al., 2008; Dess & Minor, 1996; Dess, 2000; Pliner & Melo, 1997). Within the sensation seeking model, each of these behaviours can be explained as the animal attempting to maintain their arousal at optimal levels. Sensation seekers'/ HiS rats' optimal arousal level is higher. Thus the sensation seeker/ HiS rat administers drugs, seeks new stimuli, takes risks etc. all in an effort to increase their arousal to optimal levels. On the other hand the non-sensation seekers'/ LoS rats' optimal arousal level is lower. Thus the non- sensation seeker/ LoS rat avoids all of these stimulating activities or stimuli in an effort to decrease arousal and maintain optimal levels of arousal. However, this model only holds true if results from the previous literature (flavoured liquids) are replicated in the current study (flavoured solids). Specifically, the previous literature indicates that the HiS rats prefer sweet, salty, and saccharin

flavoured liquids more so than the LoS rats, and stress more strongly affects the behaviour of the LoS rats compared to the HiS rats (Dess, 2000; Dess & Minor, 1996). If the results are not found to support the sensation seeking framework, some other factors may be at play and influencing the rats' behaviour.

1.3 Summary

In summary, the current literature on the HiS and LoS strains has established that these strains differ on consumption of saccharin, which is their defining characteristic (Nachman, 1959). Along with differing in saccharin preference, the literature indicates that the strains differ on liquid taste preferences, consumption during stress, as well as various other behaviours such as propensity for drug abuse and stress reactivity (Carroll et al., 2002; Carroll, Anderson & Morgan, 2007; Carroll et al., 2008; Dess & Minor, 1996; Dess, 2000). However, there is a gap in the current literature regarding how stress affects the taste preferences of these strains and the taste preferences for flavoured solids of these strains. The current study addressed this gap in the literature by investigating if these differences in taste preference still exist when using flavoured foods (instead of liquids). Additionally the effects of stress on these taste preferences, and on food consumption in general were also investigated.

With this end in mind, first the phenotype of the HiS and LoS strains will be defined and previous studies using these strains of rats will be summarized. Next, food preferences of both the typical rat population and the HiS and LoS rats will be discussed. This will be followed by a description of the literature regarding alliesthesia and the effect of satiety and caloric content on consumption, as well as a brief description of some of the physiological/neurological mechanisms involved. This will lead into a brief description of the stress response, followed by

literature outlining how stress affects the HiS and LoS rats differently. Once the stress response is fully understood, the literature regarding the effects of stress on consumption and food preference for both the normal rat population and the HiS and LoS strains will be outlined. Once the literature regarding consumption, taste preferences, and the effects of stress on these phenomena have been outlined, the sensation seeking/optimal arousal paradigm will be described. The sensation seeking paradigm will be used to frame the results of the previous studies as well as the predictions of this study. However, literature regarding alliesthesia will also be explored as a potential influence on the results of the current study. To begin the literature review, the characteristics of the HiS and LoS rat strains will now be discussed.

2 Literature Review

2.1 Phenotype of the HiS and LoS strains

2.1.1 Differences in Saccharin Preference

Variability of saccharin preference among the normal rat population was initially discovered in a study by Nachman (1959). This discovery then led to the subsequent breeding of the HiS and LoS strains in existence today. In the original study Nachman (1959) was attempting to study the effects of diet on saccharin preference. In the process Nachman actually observed that within his sample population hooded rats had a greater preference for saccharin than Sprague Dawley albino rats, and albino rats showed greater variation in saccharin preference. These findings led Nachman to speculate that the difference in saccharin consumption he observed was due to inherited genetic differences. In an attempt to support this hypothesis, Nachman designed a study to demonstrate the heritability of saccharin preference by selectively breeding the preferring and the non-preferring rats. The parent generation consisted of 36 Sprague Dawley albino rats that were then given daily 5-hour tests to test taste preference between water and a 0.25% saccharin solution for 26 days. The last four days of these preference tests were conducted under 14-hour hunger and thirst. Preference scores identified the rats as either extreme saccharin preferring or extreme water preferring after 1 hour during the daily tests. From here the extreme saccharin-preferring rats were bred together and the extreme water-preferring rats were bred together to produce the F1 generation. With the F1 generation, 22 daily 1-hour tests were performed to identify the high-saccharin-preferring and the low-saccharin-preferring rats. Again the high-saccharin-preferring and the low-saccharin-preferring rats were bred to create the F2 generation.

The results of this study provided evidence that saccharin preference has a genetic basis, and more generally that taste preferences are genetically influenced. The mean percentage of saccharin consumed by the high-saccharin-preferring and the low-saccharin-preferring rats of the parent generation were the same (both were *M*= 65%). However, as the rats were selectively bred, the differences between the high-saccharin-preferring and the low-saccharin-preferring rats grew with each generation. For the F1 generation, the mean percentage of saccharin consumed by the high-saccharin-preferring rats was *M*= 69% and the mean percentage for low-saccharin-preferring rats was *M*= 42%. For the F2 generation, the mean percentage of saccharin consumed by the high-saccharin-preferring rats was *M*= 82% and the mean percentage of saccharin consumed by the low-saccharin-preferring rats was *M*=40%. From this experiment Nachman provided evidence indicating that saccharin preference is heritable and it was from this experiment that high-saccharin and low-saccharin-preferring rats were first bred. Now the Occidental College Low Saccharin (LoS) and Occidental College High Saccharin (HiS) strains as well as the HiS and LoS strains from the University of Minnesota exist as a result of this initial study (Dess & Minor, 1996). Following this discovery, further research with these strains was performed in an attempt to better define the phenotypes of the HiS and LoS rats.

For example, Dess and Minor (1996) performed an experiment with the intention of determining the reaction of the HiS and LoS strains to saccharin across various concentrations. HiS and LoS rats were presented with five different saccharin concentrations (0.05%, 0.1%, 0.25%, 0.7 %, and 1.0 %) in a 24-hour two-bottle preference test with one of the saccharin concentrations and water being presented. The 24-hour two-bottle preference tests occurred consecutively until each concentration was presented to the animals five times. These concentrations were presented in ascending and then descending order. Each run was separated

by a day in which the rats had ad libitum access to food and water. During the two-bottle preference tests, avidity[1] and preference[2] scores were taken.

When using avidity scores, the preference function was an inverted U with the peak resting at 0.25 % for both runs. This means that the rats' preference for saccharin increased as the concentration approached 0.25 % and then after that point the preference decreased. The descending run showed lower avidity scores over all for both strains and the peak of the function rested at the same concentration. The strain difference in saccharin consumption remained up until 0.7%, and no strain difference was found at 1.0%. For preference scores each run showed a decrease in preference for both strains as the concentration approached 0.7%. The scores were lower in the second run compared to the first run. The strain difference remained throughout all concentrations. Therefore, when weight is being accounted for (avidity scores), consumption of a saccharin solution increases for both strains as concentration increases up to 0.25% at which point consumption decreases. Line differences (HiS consuming more saccharin solution compared to LoS) remain significant up until a concentration of 0.7%. When weight is not accounted for, preference for saccharin decreases in both strains from 0.25% up until the 0.7% concentration while strain differences remain significant.

Keeping this and other literature in mind the current study used a 0.1% saccharin solution to test the phenotype of the subjects. A concentration of 0.1% is the most commonly used concentration among the Dess and Carroll literature with the HiS and LoS rats (Dess & Minor, 1996; Dess, 2000; Carroll et al., 2008). This saccharin solution concentration is also within the range of concentrations that are preferred by both strains, while the HiS rats still have significantly greater preference and avidity scores for the saccharin solution compared to the LoS rats.

2.1.2 Differences in Stress Response

Along with saccharin preference differences between the two strains, HiS and LoS rats differed on emotionality, as measured by an open field test (Dess & Minor, 1996). In the open field test the rats were placed in an apparatus which was empty and open apart from an enclosed start box from which the subjects began each trial. During a trial food pellets were placed in the open field to entice the rats out of the start box and the animals' behaviour was monitored during the trial with regard to emerging from the start box and exploring. LoS (M=252.5 s) rats emerged more slowly from the start box than the HiS rats (M=187.5 s). These results suggest that the LoS rats are more anxious than the HiS rats. Thus along with saccharin preference, there appears to be an emotionality difference between the two strains. This makes them ideal subjects in experiments studying stress and eating; emotionality is a term referring to the observable behaviour of an emotion (for example, a smile is the observable behaviour of happiness) (Reber & Reber, 2001). Therefore, the literature indicates that the HiS and LoS rats differ in their behavioural expression, of what the investigators are interpreting as, stress (Dess & Minor, 1996). Thus the initial goal in working with the HiS and LoS strains was to examine the link between emotion and food preferences (Dess & Minor, 1996). The current study carried on with this goal, but focused on how stress affects food preferences as well as how it affects overall consumption of solid foods.

2.1.3 Differences in Drug Administration

Along with aiming to better understand the phenotype of the HiS and LoS strains, there was also an effort to study the importance of this behavioural phenotype as a model of the initial development and progression of drug abuse. This line of research began with the observation that

the strains not only differed on saccharin consumption but on ethanol consumption as well, with the HiS rats consuming more than the LoS rats (Carroll et al., 2002; Carroll et al., 2008). From this finding it was hypothesized that HiS rats may be genetically predisposed to over consume other drugs; thus self-administration of cocaine by these strains was investigated. To explore this hypothesis, four groups (HiS males, HiS females, LoS males and LoS females) were trained to self-administer intravenous cocaine for 30 consecutive days. Each session lasted six hours. Drug self-administration was considered to have occurred when 100 self-administered infusions occurred in a 6-hour session for five consecutive days. HiS rats acquired cocaine self-administration faster than LoS rats (M=13 versus *M*=26 days). This difference suggests that the HiS and LoS strains also differ in drug administration acquisition due to selective breeding (Carroll et al., 2002). This would also suggest that the propensity for drug abuse is genetically linked with the other characteristics observed in the HiS and LoS strains such as different taste preferences and stress responses.

The research has shown that these strains are useful for the purpose of studying the progression of drug abuse, as indicated by the greater propensity toward drug administration and greater impulsivity shown by the HiS strain compared to the LoS strain. These behaviours are characteristic of the initial development and progression of what may lead to drug abuse and thus the HiS strain shows great promise as a model for this stage of drug abuse (Carroll et al., 2002; Carroll, Anderson, & Morgan, 2007; Carroll et al., 2008). Compared to HiS rats, LoS rats are more fearful in an open field experiment, and less able to cope with stressors such as glycemic challenges and electric shock (which will be further elaborated upon when discussing the stress response of the HiS and LoS strains). This would make the HiS and LoS strains useful in studies

regarding stress reactivity and its various effects on the body; for example, changes in consumption (Dess & Minor, 1996; VanderWeele, Dess, & Castonguay, 2002).

2.1.4 Summary of Strain Differences

The differences discovered between these two strains suggest that these rats may exhibit traits which make them useful in research regarding stress, food consumption, drug use, and the interaction between these attributes (Carroll et al., 2002; Carroll et al., 2008; Dess & Minor, 1996; VanderWeele, Dess, & Castonguay, 2002). The literature with the HiS and LoS strains has given evidence to support the notion that stress/emotionality and taste preferences/food consumption have roots in genetic composition and are genetically linked together (Carroll et al., 2008; Dess & Minor, 1996). The previous literature would indicate that the HiS and LoS strains are appropriate test subjects for studies regarding food consumption and stress and the link between the two, which is why they were used in this study.

The current study will provide further support of the usefulness of the HiS and LoS strains as models for observing the interaction of stress and food consumption. The study also provides literature regarding the effects of stress on the food preferences of these strains, a line of research which does not yet exist. This study then offers the sensation seeking/ optimal arousal paradigm, as a possible cohesive theoretical framework within which to understand the previous literature, and from which to make predictions for the current study. Now, before the effects of stress on consumption and food preference can be addressed, the food preferences of both the typical rat population and of the HiS and LoS strains will be explored.

2.2 Food preferences of non-bred strains and the HiS and LoS strains

Like humans, rats can detect various different flavours and have been found to prefer certain flavours over others (Nami, Brand, Christensen, & Kare, 1986; Tordoff, Alarcon, & Lawler, 2008). In food preference experiments with rats, it has been determined that they can detect even small amounts of flavourings on their regular food. Rats also have a wide range of preferred flavours, many of which may have roots in evolutionary history (Myers & Scalfani, 2006; Tordoff, Alarcon, & Lawler, 2008). Often, innate food preferences are for foods which aid in survival (i.e. high caloric value, signaling the presence of nutrients) (Pinel, 2009).

The food preferences of both the normal rat population and HiS and LoS rat populations will now be outlined (Dess, 2000; Naim et al., 1986; Tordoff, Alarcon, and Lawler, 2008). First, a study investigating the preferences of the normal rat population for differently flavoured semi-purified rat chow will be described (Naim et al., 1986). This will be followed by a study detailing the taste preferences of the Sprague Dawley rat strain for differently flavoured liquid solutions (Tordoff, Alarcon, & Lawler, 2008). And lastly, a study examining the flavour preferences of the HiS and LoS strains for different flavoured liquid solutions will be discussed (Dess, 2000). The study by Naim et al (1986) regarding flavour preferences when added to semi-purified food will now be reviewed.

2.2.1 Taste Preferences of the Normal Rat Population

In an experiment by Naim et al (1986), male Sprague Dawley rats were used to determine the optimally preferred concentrations of various flavoured compounds when added to a semi-purified rat chow. The data collected for each compound used resulted in an inverted-U-preference-aversion function. This is where the amount consumed increases as concentration

increases up to a concentration where the flavour becomes unpalatable, at which point consumption decreases. The data was collected via a two-choice preference test in which two different flavoured foods were presented to the rats in two separate cups. One was the plain semi-purified rat chow as the control food and the other was the semi-purified rat chow flavoured with one of the 14 flavoured compounds. The preference was tested over a short and long duration with consumption being measured at 1 hour, and then at each 24-hour mark for 5 days. The flavours were each presented at five different concentrations ranging from 0.002% to 2.0% per flavour. Rats were able to detect even small concentrations of the various flavours added to the base diet. Nacho cheese, bacon, cheddar cheese, vanilla, peanut, chocolate, salami, liver, bread, cheese paste, chicken and beef were all preferred compared to plain semi-purified rat chow.

The data indicated that the rats tended to prefer flavours of nutritionally sound foods (peanut, chicken, beef, etc.) as compared to more "junk food" flavours (chocolate, vanilla, etc.). This suggests a possible innate preference for flavours that, if found in nature, would signal a food that is nutritious. These would be foods that have the most nutritional value such as high calorie foods and foods containing vital nutrients (Myers & Scalfani, 2006). This result provides suggestive evidence for evolutionary/genetic roots with regard to food preferences. This conclusion can be drawn because it has been found in previous literature that rats and other mammals are genetically geared to prefer flavours which indicate that a food increases their chance of survival (Myers & Scalfani, 2006).

This study is a good example of successful flavouring of rat food. This methodology allows future studies to control nutritional content and texture, by flavouring rat food, without affecting the hedonic properties of the preferred tastes. The successful results of this study

support the decision of the current study to flavour rat food with different flavoured compounds. The study also supports the use of two-choice preference tests with flavoured food and the plain unflavoured food as the control, as was used in the current study (Naim et al., 1986). To further examine taste preferences and how they differ by rat strain. Tordoff, Alarcon and Lawler (2008) examined the preference for 17 different taste compounds in water among the Sprague Dawley strain and 13 other strains of rat. This study will now be reviewed.

Tordoff, Alarcon, and Lawler (2008) investigated the preference for 17 various taste compounds by 14 strains of rats. For the purposes of the current study only the results for the taste compounds pertaining to the present study for the Sprague Dawley strain will be reviewed. The rats received seventeen series of two-bottle choice tests with five to seven concentrations of each taste solution, concentrations were chosen based upon the literature, and presented in ascending order. Each series consisted of a 48-hour test with two bottles of water (baseline period) and then between four and seven successive 48-hour two-choice tests of water and various flavoured concentrations. Total intake (mL) and preference score can be found in Table 1. The concentration that was more preferred for each solution is presented because these numbers are the basis for the concentrations chosen for the present study.

Table 1.

Mean Preference Scores and Mean Total Intake

Compounds	NaCl	Saccharin	Sucrose	Citric Acid	Quinine
Concentration	0.58%	0.24%	1.08%	0.02%	0.0004%
Preference Score	0.98	0.98	0.99	0.50	0.45
Total Intake (mL)	41	65	45	15	10

If preference is defined as a preference score greater than 0.50 then the following conclusions can be made. Sodium chloride (NaCl), saccharin, and sucrose were optimally preferred to water at concentrations of 0.58%, 0.24%, and 1.08% respectively. For citric acid and quinine hydrochloride, these solutions are aversive beginning with the lowest concentrations and becoming more unpleasant as concentration increased. The study just reviewed provides an excellent index of various taste preferences among different rat strains. With regard to the current study, the HiS and LoS rats were descended from the Sprague Dawley strain, and thus the results from this study for that strain were considered when designing this experiment. Although only the Sprague Dawley results were shown, it should be noted that there were differences in preferences between the different strains of rats. This provides further evidence for the genetic basis of food preference, and justifies the use of selectively bred rats in various consumption experiments as well as use of the HiS and LoS strains in the present study. The results of the reviewed study pertain to the current study because these are the basis for the concentrations of the various solutions chosen for the present study.

The two studies just reviewed have outlined the preferences of the Sprague Dawley strain for various flavours such as sweet, salty, and other flavours, often ones that correlate with foods that are beneficial to survival (Myers & Scalfani, 2006; Naim, 1986; Tordoff, Alarcon, and Lawler, 2008). The studies also provide evidence for successful flavouring of rat food as well as using plain food and flavoured food presented simultaneously in a two-flavour preference test (Naim, 1986). Now a study by Dess (2000) regarding the taste preferences of the HiS and LoS strains will be reviewed.

2.2.2 Taste Preferences of the HiS and LoS strains

In the study looking at the taste preferences of the HiS and LoS strains by Dess (2000), the responses of HiS and LoS rats (both male and female) to various basic taste qualities were measured. During this study, an initial baseline measurement of water intake was taken over 2 consecutive days. Following this, a series of two-bottle preference tests was performed between water and: sucrose, quinine hydrochloride, citric acid, sodium chloride, and a quinine adulteration of a sucrose solution of various concentrations. Results for each solution will now be discussed.

For sucrose, the concentrations 0.125%, 0.25%, 0.50%, 0.75%, 1.0%, 1.5%, and 2.0% were presented. Avidity and preference scores increased as concentration increased and the 2.0% sucrose solution was the most preferred and had the highest avidity scores. The HiS rats were found to have significantly greater preference and avidity scores compared to the LoS rats for each of the concentrations presented. Females were also shown to have higher avidity scores than males and there was a greater difference between the HiS and LoS females than the HiS and LoS males.

For quinine hydrochloride as well as citric acid there was no difference in avidity and preference scores between the HiS and LoS strains. For quinine hydrochloride the concentrations 1.0 mg/L, 5.0 mg/L, 10.0 mg/L and 20.0 mg/L were presented and the 20 mg/L solution was the least preferred concentration. For citric acid the concentrations 0.01%, 0.025%, 0.05%, and 0.1% were presented. The 0.1% concentration had the lowest preference scores and avidity remained constant for the most part across concentrations. The concentrations of 20 mg/L and 0.1 %, for quinine hydrochloride and citric acid respectively, were the highest concentrations presented of

these solutions, and were the most aversive to the subjects. These concentrations were used in the methodology of the current study.

For sodium chloride the concentrations 0.05%, 0.1%, 0.45%, and 0.9% were presented to the subjects. Both preference and avidity scores increased as concentration increased, with the 0.9% concentration having the greatest avidity scores, with females having higher avidity scores than males, and HiS rats having higher avidity scores than LoS rats. With regard to preference scores for sodium chloride, the highest preference was for the 0.45% concentration for both strains and sexes, and HiS preference scores were greater than LoS preference scores.

Finally, a 2.0% sucrose solution was adulterated with increasingly greater concentrations of quinine (1.0 mg/L, 10.0 mg/L, 20 mg/L, 40 mg/ L, and 60 mg/ L). Overall preference scores and avidity scores decreased as quinine concentration increased and, LoS rats' preference scores were lower than HiS rats' preference scores. The highest quinine concentration (60mg/L) used to adulterate the sucrose solution yielded the lowest preference and avidity scores for both strains and sexes.

These results provide further evidence that there are differences in taste preferences between the HiS and LoS strains. The HiS strain has greater preference scores compared to the LoS strain for sucrose and sodium, as well as higher avidity scores for these compounds. A particularly important result was the difference in avidity and preference scores between HiS and LoS strains for the quinine adulteration of sucrose. This is an important result as it provides a possible explanation for why HiS and LoS rats differ in saccharin consumption. It suggests that the reason HiS rats prefer saccharin more than the LoS rats is because the LoS strain is more sensitive to the bitter quality of saccharin. This would explain why LoS consumption decreased

more as the quinine concentration, used to adulterate the sucrose solution, increased. The difference in preferences by the HiS and LoS strains in this study further supports the notion that food preferences have some basis in genetic disposition. The results from this study regarding which concentrations were optimally preferred (sodium was most preferred at 0.9% and sucrose was most preferred at 2.0%) or were the most aversive (quinine was most aversive at 20 mg/L and citric acid was most aversive at 0.1%) were considered when choosing the concentrations used in the current study.

2.2.3 Summary of Taste Preference Literature

The literature reviewed demonstrates that rats have various foods and tastes that they prefer and do not prefer (Nami, Brand, Christensen, & Kare, 1986; Tordoff, Alarcon, & Lawler, 2008). The results suggest that these food preferences have roots in a genetic predisposition to prefer foods which help aid in survival (i.e. foods high in caloric value or nutrients). For example, the rats were found to prefer salty (associated with electrolytes) and sweet tastes (associated with carbohydrates/calories). Conversely rats find sour and bitter tastes aversive, tastes which in nature would indicate the presence of poison or indicate that a food has spoiled (Myers & Scalfani, 2006; Pinel, 2009). Further evidence for the genetic basis of food preference is that the HiS and LoS strains, which have been bred to differ in saccharin preference, appear to have also been bred to differ in the preference for other flavours as well.

However, taste preference is rarely as simple as when it is modeled in experimental methodology using flavoured liquids and two-bottle preference tests. For example, satiety and hunger have been shown in the literature to have an effect on taste preferences and consumption behaviours. This phenomenon is known as alliesthesia. Specifically, alliesthesia is when there is

a change in how one perceives a stimulus, and this change occurs based upon the perceiver's physiological state (Cabanac, 1971). The effect of satiety on taste preference and the role of the Primary Gustatory Cortex (PGC) will now be outlined. To begin, the role that the (PGC) plays in the effect of satiety on taste preferences will be detailed. The role of the vagus nerve and various hormones that play a part in the process of satiety will be described as well. This will be followed by a brief review of the main conclusion in the literature regarding how satiety effects taste preferences.

2.3 The PGC and the Influence of Satiety and Food Qualities on consumption

The PGC (located at the anterior of the insula and at the frontal operculum) is a multisensory neural network which integrates perception, emotion, cognition, and gustatory information (i.e. caloric content and texture of food) (Frank et al., 2013; Goldstein, 2010). The PGC processes the sensory perception of food, such as its taste, smell and appearance, while integrating external influences on consumption such as emotional cues and other internal cues (calories consumed etc.) (Frank et al., 2013). The PGC also receives information from the stomach by way of the vagal afferents present in the gut. These vagal afferents are stimulated by various gut hormones (cholecystokinin, Glucagon- like peptide-1, peptide YY, Amylin, etc.) which influence feelings of satiation, hunger and taste preference as well as help to limit meal size and post-meal hunger (Scalfani, 2013; Woods, 2013)

Each of the signals integrated within the PGC (calorie content/texture of food, stress, temperature of food) have been reported in the literature to influence consumptive behaviour when measured individually (de Wijk et al., 2008; Frank et al., 2013; Geliebter, 1979; Hogenkamp et al., 2012; Naim et al., 1986; Young,et al., 1974; Zijlstra et al., 2008). For

example different food textures have been found to influence consumptive behaviour (de Wijk et al., 2008; Hogenkamp et al., 2012; Naim et al., 1986; Zijlstra et al., 2008). Rats have been found to prefer small pellets of rat food when compared to powdered rat food or larger pellets (Naim et al., 1986). Additionally, the literature has also shown that intake of a liquid food product is higher compared to intake of a semi-solid food product when all other variables are equal (caloric content, palatability, eating effort/rate, etc.). It has been suggested that liquids are less satiating than solids. This has to do with the fact that liquids can be consumed quicker and spend less time in the oral cavity, which means less exposure to sensory receptors which allows less time for satiety signals to be transmitted to the PGC to indicate that the subject is full. As a result liquids are consumed in greater amounts when compared to solids despite caloric or nutritional content (de Wijk et al., 2008; Hogenkamp et al., 2012; Zijlstra et al., 2008).

The literature has also shown that the caloric content of food influences consumptive behaviour as well (Geliebter, 1979; Young et al., 1974). For example, sham fed rats (a gastric fistula allows for chewing and swallowing of food but redirects food away from stomach and out of the body) have been found to continue to eat for up to 17 hours after initiation of testing without becoming satiated. In contrast, during normal consumption with the gastric fistula closed (thus food makes it to the stomach) the rats have been shown to eat for only the first 15 minutes of a trial (Young et al., 1974). So even if the act of eating occurs, if the food does not reach the stomach and the calories are not absorbed satiety is not reached. This demonstrates that the presence or absence of calories has a significant influence on consumptive behaviour and satiety. Other studies have shown a similar result when using non-caloric food stuffs. Non caloric food stuffs (kaolin and water mixture) do not suppress appetite when compared to caloric food stuffs, despite there being a volume of liquid present in the stomach. This provides evidence that

calories are necessary for satiety and that the caloric content of a food influences consumptive behaviour, specifically that consumption of a caloric load differs compared to consumption of a non-caloric load (Geliebter, 1979).

The consumption of calories, and the hormonal changes that result, have also been found to influence taste preferences. For example, a study by Holman (1969) was one of the first studies which demonstrated that food preferences can be influenced by the post-oral action of nutrients. This was demonstrated by a classical conditioning paradigm. The conditioned stimulus (CS) was an aversive flavoured solution and the unconditioned stimulus (US) was an intragastric infusion of a substance high in fat and sugar (i.e. eggnog). After the animals were presented the CS (a sour solution) and the US paired together their taste preferences were then tested for the CS+ (where the US is present) and the CS - (a bitter solution with the US not present). It was found that the rats significantly preferred the CS+ as compared to the CS – as was determined during a two- bottle preference test. This result suggests that the nutrients of the eggnog infusion, and the resulting changes in gut hormone secretions, caused a change in the taste preferences of the subjects towards the taste associated with the delivery of calories (Holman, 1969).

Generally speaking, the literature has found sensory specific satiety to occur; this is when preference for and consumption of a various food stuff decreases when it has already been recently eaten. This effect has been measured by changes in behaviours (such as positive hedonic behaviours in response to tasting a stimulus), changes in consumption amount, as well as changes in self report measures of preference (Berridge, 1991; Griffioen et al., 2012; Moskowitz et al., 1976; Rolls et al., 1988). This sensory specific satiety effect has been demonstrated during the consumption of foods containing various nutrients, as well as when using intragastric infusions of sucrose, milk, and other nutrients (Berridge, 1991; Griffioen et al., 2012; Moskowitz

et al., 1976; Rolls et al., 1988). Another noteworthy conclusion is that sated subjects were found to demonstrate a decreased preference for different foods/flavours, as compared to hungry subjects. This effect was particularly pronounced when the subjects were satiated by a high protein, high starch, or high sucrose food stuff (Laeng et al., 1993; Berridge, 1991; Griffioen et al., 2012; Moskowitz et al., 1976; Rolls et al., 1988).

The above conclusions regarding satiety, taste preference and food qualities suggest that the qualities of what is consumed, as well as the physiological state of an individual, may affect taste and food preferences (Berridge, 1991; de Wijk et al., 2008; Geliebter, 1979; Griffioen et al., 2012; Hogenkamp et al., 2012; Holman, 1969; Laeng et al., 1993; Moskowitz et al., 1976; Naim et al., 1986; Rolls et al., 1988; Young et al., 1974; Zijlstra et al., 2008). These dynamic changes in taste preference are the result of changes in gut hormone levels and, as a result, changes in activation of the PGC by vagal afferents in the gut (Frank et al., 2013; Goldstein, 2010; Scalfani, 2013; Woods, 2013). The current study must keep in mind the effect that calories and nutrients may have on taste preferences and consumptive behaviour when interpreting the results as this study uses flavoured foods (solids with calories). This is different than in the previous literature, where flavoured liquids (liquids with no calories) are used when determining the taste preferences of the HiS and LoS strains. Because calories and texture of food have been found in the previous literature to influence consumptive behaviour, it is logical to posit that a combination of these variables may have a cumulative effect on consumption as all this information is integrated within the PGC (de Wijk et al., 2008; Frank et al., 2013; Geliebter, 1979; Hogenkamp et al., 2012; Naim et al., 1986; Young, et al., 1974; Zijlstra et al., 2008). Now that the main conclusions from the satiety and taste preference, and the food quality literature have been elaborated upon, the acute stress response and its effects on the body will now be

reviewed. First the general physiological processes of the stress response will be outlined, after which the literature regarding the acute stress response of the HiS and LoS strains will be discussed.

2.4 The Acute Stress response

The physiological changes characteristic of the acute stress response act as an adaptive response to what the body perceives as a threat (Comer, 2010). The acute stress response involves neurological, sympathetic nervous system, and endocrine system changes. When an organism experiences a stressor an alarm reaction in the brain initiates the stress response by first identifying a stressor. This results in increased alertness and an increased metabolic and circulatory rate of the organism (Eriksen, 1999). The acute stress response operates within two pathways in the brain, the Hypothalamic-Pituitary-Adrenal Pathway (HPA) and the sympathetic nervous system. When the HPA axis is activated, the periventricular nucleus of the hypothalamus releases corticotropic releasing hormone (CRH) which acts upon the pituitary gland which secretes adrenocorticotropic hormone (ACTH). ACTH then causes the adrenal cortex to release other corticosteroids. ACTH and the other corticosteroids of the HPA axis act upon various organs in the body resulting in arousal such as increased heart rate, breathing rate, and blood pressure (Brunton, Russell, & Douglas, 2008; Comer, 2010; Yehuda & Mostofsky, 2005)

When the sympathetic nervous system is activated the adrenal medulla releases epinephrine and norepinephrine. These hormones act upon various organs, resulting in a fear/arousal response. Symptoms of sympathetic nervous system activation include dilated

pupils, inhibited salivation, relaxed bronchi, accelerated heart rate, inhibited digestion, and relaxed bladder (Comer, 2010).

The acute stress response is the body's compensatory response to a perceived threat (Eriksen, 1999). Some symptoms of this response include increased heart rate, breathing rate, blood pressure, dilated pupils, inhibited digestion/salivation, relaxed bronchi and relaxed bladder (Brunton, Russell, & Douglas, 2008; Comer, 2010; Yehuda & Mostofsky, 2005). Rats, specifically when stressed, can exhibit certain behavioural symptoms that indicate the animal is distressed. These behaviours may include a change in movement from baseline (an increase or decrease in movement or exploration), increased grooming, being frozen or not moving, increased licking behaviour, increased exploration, as well as increased defecation and urination (CCAC, 2003).

Additionally, many of these changes in the body during the stress response are adaptive (Pinel, 2009). The inhibited digestion that occurs during the sympathetic nervous system response, for example, occurs so that resources may be re-directed to the muscles to make energy available in a fight or flight situation (Comer, 2010; Yehuda & Mostofsky, 2005). Therefore it is logical that animals may change their eating behaviour in order to store and gather energy in response to a threat. This may be accomplished by the animal exhibiting an increase in consumption in order to have more energy available. Or, due to the inhibited digestion that is characteristic of the sympathetic nervous system response, the animal may reduce its feeding behaviour and focus on coping behaviours such as grooming, the freezing response, exploration, etc. (CCAC, 2003). Now that the acute stress response has been elaborated upon, the stress response of the HiS and LoS strains will be described.

2.5 The Stress Response of the HiS and LoS strains

In the HiS and LoS strain of rats, a study by VanderWeele, Dess, and Castonguay (2002) investigated the endocrine changes, specifically changes in corticosterone level, during the stress response of these two strains. To measure the blood corticosterone levels, blood samples were taken from the animals' tails and analyzed. These samples were taken in the morning (AM) and the evening (PM) as baseline measures as well as after the stressor. The stressor used on the HiS and LoS rats was a tail shock in which the animals were exposed to 60, 0.6 mA shocks of a duration of 5 seconds each. The goal of this study was to induce stress and to then examine differences between the resulting corticosterone levels of the two strains. When comparing baseline corticosterone levels of the HiS and LoS rats from AM and PM that LoS (*Mam*=100 ng/ml; *Mpm*=375 ng/ml) rats had a significantly higher baseline corticosterone level than the HiS rats (*Mam*=50ng/ml*; Mpm*=275 ng/ml). When the post stress corticosterone levels were compared between the strains, it was again found that the LoS rats (*M*=535 ng/ml) had a higher cotricosterone level than the HiS rats (*M*=400 ng/ml); however this difference only approached significance ($p = 0.064$).

These results suggest that the LoS rats may exhibit a stronger physiological response to a stressor; however, the results did not quite reach significance. The results do provide evidence that the HiS and LoS strains differ in baseline corticosterone levels, suggesting that the two strains have differing baseline arousal levels. Thus further testing of the HiS and LoS strains reactions to stress would be useful in determining if there are actual differences in how the HiS and LoS rats react to stress. Further experimentation would help to determine if these results are a true representation of the stress response of the strains. This is where the present study is useful as it explored symptoms of the stress response (changes in eating). If there are differences in this

eating behaviour of the strains in response to stress, then there is likely a corresponding difference in the physiological stress response. The results also showed that the LoS rats were more anxious, even without the stressor, than the HiS rats, as indicated by the greater corticosterone levels at baseline. Thus it appears that the LoS animals are more anxious and slightly more reactive to stress (though not significantly).

In the same study, another experiment was performed to determine if the HiS and LoS rats differ in their response to a glycemic challenge. Specifically, they examined the feeding behaviour of the HiS and LoS rats in response to both slow and rapid onset glucoprivation (which is a lack of utilizable glucose). This was accomplished by injecting the two strains with regular insulin, fast acting insulin, 2- DG (a fast acting drug which acts similarly to insulin) and a saline solution as a control. Then the resulting feeding behaviour was monitored. The strains were not found to differ in their rat food consumption in response to the saline or the slow acting insulin. However, the LoS (M_{Insulin}= 0.1 g/hr; $M_{2\text{-}DG}$= 0.1 g/hr) rats were found to have a slower eating rate during the first half hour after receiving the fast acting insulin and the 2-DG as compared to the HiS rats(M_{Insulin}= 2.0 g/hr ; $M_{2\text{-}DG}$= 4.6 g/hr). This result supports the notion that the LoS rats are less efficient at responding to a metabolic stressor compared to the HiS rats. Thus the HiS and LoS strains differ in their responses to a metabolic stressor.

These results provide evidence that there is variation in the response of the two strains to an internal metabolic stressor, as well as a variation in corticosterone levels certainly before and possibly after stress between the two strains. However, there is more than one possible explanation for these results. For example, the LoS rats may not have detected the change in blood glucose levels as quickly as the HiS rats, thus resulting in a slower reaction to the stressor.

Conversely, it is possible that the HiS rats over reacted to the lower blood glucose levels resulting in the quicker eating rate of the HiS rats compared to the LoS rats.

Thus it appears that in the process of selecting for taste preference differences (saccharin preference), differences in stress reactivity were also selected for. These results support the sensation seeking/optimal arousal paradigm which was used to frame the hypotheses of this study, and which will be elaborated upon later. If it is true, as the literature has indicated, that HiS and LoS rats react differently on a hormonal level to acute stress (as indicated by different blood corticosterone levels), then it is also logical to assume that HiS and LoS rats may change their eating behaviour in response to a stressor differentially (Dess & Minor, 1996; VanderWeele, Dess, & Castonguay, 2002). Now that the stress responses in the normal and HiS and LoS populations have been examined, the effects of stress on eating behaviour will be presented.

2.6 Effects of Stress on Consumption (normal population)

Previous research demonstrates that stress has differential effects on food consumption depending on the type of stress used and experimental situation (Krebs et al., 1996; Macht et al., 2001). For example, electric shock, tail pinch, or social conflict have been shown to result in increased eating during stress whereas chronic noise, restraint stress, and cold swim stress result in decreased eating (Krebs et al., 1996). This literature will now be examined with regard to the normal population and the following section will cover stress-related changes in consumption of the HiS and LoS strains.

2.6.1 Decreased consumption during times of stress

In a study by Krebs et al (1996) the effects of stress on eating behaviour were investigated. For the study they used naïve male Sprague-Dawley rats, and compared between subjects for the stress condition where the rats were exposed to a 95 dB white noise stressor, and the control condition where the rats were exposed to a 60 dB white noise stressor. During these conditions, food intake, duration of feeding, speed of eating behaviour and defecation rate were measured. The stress group was exposed to 5 sessions of stress with the 95 dB noise stressor. Initially food intake in the stress group decreased to a mean of 50 pellets, while the control condition had a mean of 75 pellets. However as the stress sessions continued, the difference in food intake between the control and stress group gradually decreased until by the final session the stress group had a mean intake of 76 pellets, equaling the intake of the control group. The rats in the stress group ate more quickly within a smaller time frame, resulting in their consumption equaling that of the control group. The mean eating rate for the stress group was 6.1 pellets per minute, and the control group had a mean eating rate of 4.9 pellets per minute, on the final testing session. Compared to the control group, the stress group was also found to exhibit increased rates of defecation, exploration, grooming, and resting.

This study indicates that acute and chronic stress affects consumption differently and also provides an example of a situation in which a decrease in consumption in response to an acute stressor would occur. At the beginning of the study, the stressed rats exhibited a decrease in consumption, as was predicted. However as the study went on, the rats began to consume more food and at a faster rate. It was hypothesized by the authors that the stress became chronic as it was repeatedly administered to the rats for 6 days. As the stress became chronic the rats acclimatized to the stress condition and changed their behaviour (increased eating speed) in order

to meet their caloric needs and consume the amount of food they normally would without the stressor present. However, these results should be generalized with caution. The rats restricted their consumption to 75% of their baseline daily food intake for the duration of the experiment. This level of food deprivation introduces a confound of extra stress that the researchers had not accounted for. The current study employed an acute stressor as well and the consumption of the subjects in this study was predicted to decrease in response to the stressor.

In a follow up to the previous study, Krebs, Weyers, Macht, Weijers, and Janke (1997) examined videos from the previous experiment to explore differences in eating speed and scanning behaviour during control and stress conditions. During the stress condition various behavioural changes were noted in comparison to the control condition. The stressed rats exhibited increased eating speed, increased scanning behaviour while eating, and were found to interrupt their eating by leaving the area. These behavioural changes during the stress condition led to the conclusion that the intense noise was interpreted as a threatening stimulus to the rats. It was rationalized that this was the case because the behaviours exhibited during the stress condition would be seen as adaptive if they occurred in dangerous environments. This supports the theory that the stressed rats' consumption decreased because the coping behaviours, such as increased scanning behaviour and leaving the eating area, became a higher priority than eating, and thus more time and effort was dedicated to coping than to feeding. The current study, in line with the results of the study just reviewed, monitored coping behaviour of the animals. It is possible that if food consumption decreased in the subjects of the current study, it may be due to coping behaviours interfering with feeding behaviour.

These two studies by Krebs et al (1996) and Krebs et al (1997) provide evidence for experimental situations in which exposure to an acute stressor resulted in decreased feeding

behaviour. The results indicate that this decrease may be the result of coping behaviours and that if the stress is applied over a prolonged period of time it will become a chronic stressor and the reduction in feeding time is offset up faster eating. It has been theorized that consumption decreases during times of stress because coping behaviours take precedence over eating. During stress, the body's main concern is using the available resources to re-establish homeostasis and/ or keep the organism safe (Krebs et al., 1996; Lovallo, 2005). For example, during the sympathetic nervous system response to a stressor, less important functions such as digestion and salivation are decreased, and resources are instead directed to the muscles. Redirecting resources to the muscles can increase chances of successfully fighting a threat or fleeing from the threatening situation (Comer, 2010). Thus there is some credence to the notion that a decrease in consumption during stress is adaptive. Now that decreased eating during stress in the literature has been outlined, increased eating during stress will be discussed.

2.6.2 Increased consumption during times of stress

In a study by Ulman, (1952), the purpose was to provide evidence for overeating in response to stress (a mild electric shock) as a means of reducing tension. To test this, female Lashley strain rats were assigned to groups which differed in how much habituation was allowed to the experimental apparatus, the intensity of shock (high=30 mA; low= 24.5 mA), and the hunger state of the animals. There were four groups with five days of training and within these four groups there was one group with high hunger and high shock intensity, a group with low hunger and high shock intensity, a group with high hunger and low shock intensity and a group with low hunger and low shock intensity; the same was true of the four groups with one day of training. Hunger levels were maintained by controlling body weight, the high hunger groups had their body weight reduced by 20% via dietary control and the low hunger had their body weight

reduced by 10% to ensure they were hungry for the procedure. For each group there were four daily sessions of shock during which their feeding behaviour was monitored. After the final shock procedure, the animals were then, for four days, fed immediately before being placed in the apparatus to ensure complete satiation. The means of pellets eaten for each group during the shock procedure are displayed in Table 2.

Table 2.

Number of Pellets Eaten During Shock Interval

Five Days of Training				One Day of Training			
HHHS	LHHS	HHLS	LHLS	HHHS	LHHS	HHLS	LHLS
225	15	46	16	309	141	107	40

Note. HHHS= High hunger high shock; LHHS=Low hunger high shock; HHLS= High hunger low shock; LHLS= Low hunger low shock.

The animals with one day of training ate more during shock, and that those with higher hunger ate significantly more than those with low hunger. Also with other variables kept constant, rats exposed to higher shock ate more than those exposed to lower shock. Thus the results from this study indicate that a moderate-level electric shock is an effective stressor, and that if this stressor is high enough it results in an increase in consumption. This may be because eating has been shown to be stress reducing, and thus the animals in this study were using eating as a means of coping with the unpleasant arousal elicited by the shock (Krebs et al, 1996). It is possible that eating when stressed is actually rewarding, as there is literature suggesting that opioid release is correlated with eating during stress, thus activating reward pathways in the

brain. This can make eating during stress a rewarding experience, thus providing motivation to do so (Adam & Epel, 2007).

Another study that demonstrated increased consumption during stress was by Hagan, Chandler, Wauford, Rybak, and Oswald (2003); they investigated the effect of binge triggers (a highly palatable food) on binge eating during stress in Sprague-Dawley rats. The rats were divided into groups, a non-restricted no-stress group, a non-restricted stress group, a restricted no-stress group, and a restricted-stress group. The restricted groups of rats were cycled through restricting and re-feeding/ stress cycles to emulate an individual with bulimia. During re-feeding the rats were given full access to their rat chow and to a highly palatable food. Food intake then was restricted and they were stressed using a foot shock. The results indicated that overeating did not occur during sessions when just the stress condition was present. For overeating to occur both a taste of a highly palatable food and the stressor had to be present. Under these conditions the intake increased by160%. Overeating was also found to occur when the subjects were food deprived. This overeating was even more exaggerated when food deprivation was coupled with stress. These results support the notion that individuals are most likely to overeat when they are stressed if they are firstly dieters, and secondly if a highly palatable food item is eaten while the individual is also stressed. This study used rats that were subjected to circumstances which were intended to make a special population (the restriction/ re-feeding cycles), a model of a human dieter, or a bulimic; thus these results should be generalized with caution. These results provide evidence that increased eating can occur in response to stress within certain parameters.

The literature reviewed provides evidence of increases in eating behaviour during times of stress. It has been theorized that this behaviour may occur because the act of eating itself has been found to be stress reducing (Krebs et al., 1996). Individuals are more likely to overeat

during stress if they are dieting. The research supports that this overeating during stress is motivated more by a reward system than by caloric needs (Hagan et al., 2003). It is also possible, as was mentioned earlier, that stress overeating is rewarding, as the consumption of palatable foods during stress has been linked with the release of chemicals involved in the reward pathways of the brain. Therefore, if eating during stress is chemically rewarding, it is likely to become a behaviour that is repeated under the appropriate circumstances, such as in the presence of highly palatable food (Adam & Epel, 2007; Hagan et al 2003).

It has been suggested that the reason the literature shows both an increase and a decrease in stressed consumption is due to how it has been operationalized differently in experiments (Krebs et al., 1996). For example, electric shock experiments and noise stressor experiments of different intensities have yielded varying results; moderate electric shock has been shown to cause increased food consumption and high intensity shock has been shown to decrease consumption (Krebs et al., 1996); the study by Ulman (1952) reviewed earlier, however, even contradicts this data where a high intensity shock resulted in increased consumption. This discrepancy may also be due to other characteristics of the experiment such as deprivation levels of the subjects or availability of certain types of foods (Hagan et al., 203; Krebs et al., 1996; VanderWeele, Dess & Castonguay, 2002; Yehuda & Mostofsky, 2005). Decreased eating, on the other hand, may occur because feeding behaviour is no longer a priority during the fight or flight response (Lovallo, 2005). This postulation is supported by the study in which decreased feeding was found to occur along with exploratory and grooming behaviours which interfered with normal consumption patterns (Krebs, et al., 1996). The eating response may also be impaired due to the physical symptoms of the stress response such as inhibited digestion and salivation (Pinel, 2009).

The literature reviewed demonstrates that the stressor itself can affect whether the rats eat more or less during stress (Hagan, Chandler, Wauford, Rybak, & Oswald, 2003; Krebs et al., 1996; Krebs et al., 1997; Ulman, 1952). Thus the choice of stressor must be taken into account when making predictions with regard to one's own experiment. For the present study a restraint stress apparatus was employed and thus decreased consumption is more likely to be observed, as evidenced in the previously reviewed literature (Krebs et al., 1996; 1997). Now that the effects of stress on the consumption patterns of the normal rat population have been explained, the literature with regard to how stress affects food preferences of the normal rat population will be described in detail. This will then be followed by a description of the changes in consumption and preference patterns of the HiS and LoS strains in response to stress.

2.6.3 Food preferences under stress

Along with the literature indicating that different stressors result in both increases and decreases in food intake, there has also been evidence of a shift in food preference as a result of stress. For example, preferences for sweet and salty foods in times of stress have been cited in the literature, as well as changes in preference for saccharin and citric acid (Dess, 1992; Christiansen et al., 2011; Tarjan & Denton, 1991; Howell et al., 1999). Studies by Dess (1992), Christiansen et al (2011), Howell et al. (1999) and Tarjan & Denton (1991) regarding stress and food preference of the normal rat population will now be reviewed.

In a study by Dess (1992) the effects of a tail shock on consumption of sucrose and saccharin in Holtzman rats was investigated. First a baseline intake of water was measured; then the rats were separated into control and stress groups. The stress group was shocked and then afterwards given access to either water and a 0.1% saccharin solution, water and a 1% sucrose

solution, or water and a 2% sucrose solution. Consumption was measured from the end of the stress session to 1600 and then daily at 1200 thereafter. Over the long term, the shocked rats drank less when saccharin was available versus when sucrose was available, of either the 2% or 1% concentrations for the first two days after the stressor was presented. Saccharin consumption was different from 2% sucrose consumption for all of the six days that consumption was measured. The values can be seen in Table 3. The author of this paper suggested that the different taste properties of sucrose and saccharin resulted in the differences in consumption after stress. For example, stress may have made the subjects more sensitive to saccharin's bitter quality and thus resulted in the decreased consumption. This postulation has been supported by other research in that laboratory (Dess, 1993). These results pertain to the current study as it suggests a mechanism behind the strain difference of the HiS and LoS strains, as well as providing an experimental example of stress affecting taste preference.

Table 3.

Milliliter Change in Consumption from Baseline Water Intake

Solution	Saccharin (0.1%)	Sucrose (1.0%)	Sucrose (2.0%)
Days			
1	4	13	20
2	8	18	22
3	9	13	22.5
4	6.5	13.5	22
5	7.5	12	21.5
6	9	11	19.5

As the previous study indicated, different flavour preference, such as preference for sucrose or preference for saccharin, may be affected during times of stress (Dess, 1992). For example, it has been cited in the literature that sucrose consumption increases in times of stress (Christiansen et al., 2011). It has been postulated that the reason for this consumption change during stress is that sucrose consumption may result in decreases in plasma corticosterone levels, as a result of an attenuation of the HPA axis response, which makes sucrose consumption rewarding (Christiansen et al., 2011). In a study by Christiansen et al. (2011) Male Long Evans rats were given free access to water and food, and were twice daily given access to 4 mL of a 30% sucrose solution (in the experimental group), or water (in the control group) for 14 days. Then groups of rats were exposed to restraint stress for 20 minutes, during which blood was taken at the beginning and end of the stress exposure. The stress was administered to certain groups of rats at different times after the last day of sucrose drinking. Stress was administered at either 1, 6, or 21 days after cessation of sucrose drinking. After presentation of the stressor a blood sample was taken and the animals were sacrificed and their brains prepared for analysis. Rats exposed to stress 1 day after the sucrose drinking had significantly less plasma corticosterone in their blood samples compared to the control group. The rats exposed to stress 6 and 21 days after sucrose drinking had significantly lower plasma corticosterone levels compared to the control by M=15%, and M=21%, respectively. The results support the notion that sucrose does decrease corticosterone levels and thus this may be a motivating factor in its consumption during times of stress (Christiansen et al., 2011). With regard to the present study, these results suggest that predicting an increase in consumption of sucrose during stress would be accurate.

In another study examining food preferences during stress in male Sprague Dawley rats, Howell, et al. (1999) the stressor was physical restraint. After exposure to the stressor, rats were

given a two-bottle preference test with water and one of another four solutions (2.5 mM (0.05%) saccharin solution; 100 mM (0.58%); NaCl solution; 0.75 mM (0.027%) quinine solution; 5.0 mM (0.1 %) citric acid solution) for at least 48 hours. Measurements of total fluid intake and preference scores were taken and compared to baseline intake of the various solutions which were taken prior to the stress condition. Results show that saccharin fluid intake was significantly reduced by stress (M_{pre}=18.0; M_{post}=16.3); however, there was no effect on preference scores. No effect on intake or preference scores of NaCl as a group was found. It was found, however, that individual salt intake increased in three rats, salt intake stayed the same in four others, and salt intake decreased in four rats. Stress did not affect intake or preference for quinine. Stress did, however, result in a significant decrease in preference for citric acid (M_{pre}=0.12; M_{post}=0.04) and in intake of citric acid (M_{pre}=5.0; M_{post}=1.7,). The values were small in the control condition so there were small effects, so the noted decrease may have just been due to repeated exposure.

These results give evidence for changes in food preferences when under stress. It is possible that the changed preference for saccharin was due to an increased sensitivity (due to the stress) to the bitter properties of saccharin, thus resulting in decreased intake (Dess, 1993; Dess & Minor, 1996). With regard to the change in response to citric acid, it is possible that the stress put the animal into a fight or flight response, resulting in heightened awareness. This led to an even greater reduction in intake and preference for citric acid, a solution which is already innately aversive (Miller, 2008). With regards to the current study, these results were considered when hypothesizing how stress will affect the consumption of various flavours by the HiS and LoS strains in the current study, as citric acid, quinine, saccharin and sodium chloride are all being used. This study also employed restraint stress to stress the animals, and this is pertinent to

the present study because it also used restraint stress. The results of this study indicate that restraint stress is an effective method to stress animals with the intention of influencing taste preference. The intention of the present study was to provide evidence of stress affecting the taste preferences of the HiS and LoS strains in particular.

Tarjan and Denton (1991), using male and female wild rabbits investigated the effects of various stress hormones, corticotropic releasing factor (CRF) and adrenocorticotropic hormone (ACTH) and their relation to sodium consumption/sodium levels during stress. Recall that CRF and ACTH are released when the HPA axis is activated (Pinel, 2009). In the study by Tarjan and Denton (1990), CRF and ACTH were systemically administered into the brains of the rabbits via intracerebroventricular infusion. These infusions lasted for 22 hours. A 0.88% saline solution was made available to the rabbits during the infusion, along with a magnesium chloride and a potassium chloride electrolyte solution of varying concentrations. For the duration of the infusion of CRF, a 5 to 7 fold increase of daily intake of the sodium chloride solution was found as compared to the control condition. An increase was also found with the infusion of ACTH. There was no change in consumption for the other electrolyte solutions. These results indicate that the biochemical changes present in the stress response result in an increased intake of sodium. The increase in sodium intake, as stress hormones increase, points to a possible role of sodium in either the stress response, or in coping with the bodily changes associated with the stress response, such as increased heart rate, increased blood pressure, changes in hormone levels, etc. (Pinel, 2009). That is, the biological need for sodium may increase when an organism is stressed. This increase may be due to the role sodium plays in the bodily changes seen with the stress response. Thus when the subjects of the current study are being exposed to stress, they may increase their sodium consumption due to biological changes. The LoS rats have been found

to have higher corticosterone levels both at baseline and in response to a stressor (VanderWeele, Dess & Castonguay, 2002). Thus it is possible they may increase their consumption of sodium during stress more than the HiS rats increase their sodium consumption during stress.

The previous section outlined the effects of stress on the consumption patterns of rats in the normal population with regard to both changes in amount consumed and changes in preference in response to stress. The effect of stress on consumption can vary depending on experimental conditions, with some situations resulting in an increase in consumption and others resulting in a decrease in consumption. These experimental conditions include the type of stressor used and the types of food/ flavours made available during stress (Hagan, Chandler, Wauford, Rybak, & Oswald, 2003; Krebs et al., 1996; Krebs et al., 1997; Ulman, 1952). For example, restraint stress results in decreases in consumption and binge eating is triggered when highly palatable food is present (Krebs et al., 1996; Hagan, Chandler, Wauford, Rybak & Oswald, 2003). With regard to preference changes during stress, sucrose and sodium consumption have been found to increase during stress whereas citric acid consumption has been shown to decrease and quinine consumption has been shown to remain the same (Christiansen et al., 2011; Dess, 1992). The increases in sodium and sucrose consumption in response to stress have been hypothesized to be a result of their mediating effects on the HPA axis response, which can be rewarding as it ameliorates the negative arousal associated with the stress response (Christiansen et al., 2011; Tarjan & Denton, 1991). Now that the effects of consumption patterns on the normal rat population have been reviewed with regard to changes in amount consumed as well as taste preferences, the same literature regarding the HiS and LoS strains will be explored.

2.7 Effects of Stress on Consumption (HiS and LoS)

The literature regarding stress and consumption in the normal population is extensive, with many underlying and often conflicting theories and findings. The literature regarding the HiS and LoS consumption during stress on the other hand, is limited. Research has shown that the HiS and LoS rats change their consumption differentially when under stress and little has been found regarding preference changes under stress (Dess & Minor, 1996). The literature on the effects of stress on the consumption patterns and food preference of the HiS and LoS strains will now be outlined.

2.7.1 Changes in amount consumed

Dess and Minor (1996) conducted a study regarding the effects of stress on food consumption of the same HiS and LoS strains being used in the current study. For the experiment, the HiS and LoS rats were stressed via tail shocks (0.6 mA) while being restrained in a plastic restraint tube. They were then returned to their home cage and their consumption of a bottle of saccharin (0.10%) and a bottle of water was monitored; food intake was monitored as well (only the food intake results will be reported here as the preference changes will be reported later in the appropriate section). Intake was monitored immediately after the stress session and then for 3 days following exposure to the stressor. These measurements were then compared to pre-stress measures of these variables which were measured for 48 hours before the stress session. Initially, stress significantly reduced food intake of the LoS rats ($M_{change\ in\ food\ intake}$= -6.25 g) and not the HiS rats ($M_{change\ in\ food\ intake}$= -2.50 g). However, there was a significant day by stress interaction for the LoS rats (F (2, 36) = 7.37), and as the days went by this decrease in consumption lessened. The result of this experiment provides evidence for differential changes in

eating behaviour in response to an electric shock stressor between the HiS and LoS strains. These differences may be due to the differential reactions to stress exhibited by the HiS and LoS rats as mentioned earlier (Dess & Minor, 1996; VanderWeele, Dess, & Castonguay, 2002). With regard to the present study, the hypotheses were informed by this data regarding a prediction of how the rats will change their food consumption during stress. Next, the effects of stress on food preferences will be addressed.

2.7.2 Effects of stress on food preferences

Another portion of the Dess and Minor (1996) study just discussed observed how stress affected saccharin preference among the HiS and LoS strains. As before, the HiS and LoS rats were exposed to a tail shock and then their saccharin, water and food intake were monitored; this occurred for three days (Dess & Minor, 1996). Stressed rats drank less avidly (had lower avidity scores) than non-stressed controls; however no interaction between strain and stress was found (Stress *M*= 7.17, Control *M*= 10.67). This study shows no difference between the strains regarding a change in flavour preference. The present study elaborated upon these results by using a larger variety of flavours as well as using flavoured food as opposed to flavoured liquids. Results using solid foods and more than one flavour would provide a more accurate analog of human eating behaviour, and thus the results can be generalized to humans with greater confidence.

Currently the literature for the food preferences of HiS and LoS rats during stress is limited. For example, the study just reviewed did not yield any strong results in support of a change in food preferences during stress and not many flavours were tested with regard to food preference changes during stress (Dess & Minor, 1996). For the literature on non-bred strains,

there have been citations of increases in consumption of sodium liquids, and decreases in consumption of saccharin, sucrose and citric acid (Howell, et al., 1999; Tarjan & Denton, 1991). The current study hoped to obtain data regarding how stress influences food preferences among the HiS and LoS strains, which can help to further characterize the strains.

2.8 Summary of the Effects of Stress on Consumption

The literature regarding the effects of stress on consumption in both the normal population as well as the HiS and LoS strains has now been reviewed. The experiments just outlined revealed that there exists a discrepancy in the literature for the normal rat population and there is a lack of literature for the HiS and LoS rats regarding changes in consumption during times of stress. Research with the normal population shows conflicting evidence for what changes occur in consumption during stress (Krebs et al., 1996). At times stress increases consumption and at other times it decreases consumption. This has been found to depend upon experimental conditions (Hagan, Chandler, Wauford, Rybak, and Oswald, 2003; Krebs et al., 1996; Krebs et al., 1997; Ulman, 1952). Although experiments with the HiS and LoS strains are lacking, what has been found is that the LoS rats will initially reduce their food intake during stress and the HiS rats consumption stays the same (Dess & Minor, 1996). With regard to preference, any changes in preference for the HiS and LoS strain are unclear and limited, whereas the normal population has been found to prefer sweet and salty things during times of stress (Christiansen et al., 2011; Dess 1992; Dess & Minor, 1996; Hagan, Chandler, Wauford, Rybak & Oswald, 2003; Tarjan & Denton, 1991).

In summary of the literature regarding the HiS and LoS strains, these strains are specially bred rats that are often used to investigate the relationship between stress/emotionality, food

preference/consumption, and impulsivity/drug abuse (Carroll et al., 2002; Carroll, Anderson, & Morgan, 2007; Dess & Minor, 1996; VanderWeele, Dess, & Castonguay, 2002). What most pertains to the current study regarding these rats is that LoS rats have been shown to be more biochemically reactive to stress (greater corticosterone levels), have differential food preferences, and decreased food intake in response to stress as compared to HiS rats (Carroll, Anderson, & Morgan, 2007; Dess & Minor, 1996; VanderWeele, Dess, & Castonguay, 2002). It has been theorized that the cited differences in eating responses (food preference and amount consumed) to stress by non-bred strains of rats may be due to differential reactions to stress (for example grooming as a method of coping versus eating as a method of coping). These differences may be dependent upon environmental factors and qualities of the stressor used (Hagan et al., 2003; Krebs et al., 1996).

There is, however, only a small amount of literature regarding the effects of stress on consumption of different foods in HiS and LoS rats, and how stress affects the amount and type of food consumed by this population (Dess & Minor, 1996; VanderWeele, Dess, & Castonguay, 2002). Thus the current study fills a void in the literature by investigating the effects of stress on the amount and type of food consumed in response to stress of the HiS and LoS strains. The current literature also lacks studies regarding consumption of flavoured solid food in this population. Thus the purpose of this current study is to investigate the effects of stress on consumption and taste preferences of the HiS and LoS strains; based upon the literature regarding the HiS and LoS strains the predictions of this study were framed within the sensation seeking/optimal arousal paradigm which will now be described.

2.9 The Sensation Seeking/ Optimal Arousal Paradigm

The characteristics outlined regarding the HiS and LoS strains draw a parallel with other breeds of rat which have been used to model sensation seeking/optimal arousal. The optimal arousal theory of motivation suggests that individuals will change behaviour accordingly to maintain a particular level of arousal which is considered optimal. The drive to maintain optimal arousal has been theorized as the mechanism behind sensation seekers and non-sensation seekers (Blanchard, Mendelsohn & Stamp, 2009; Pliner & Melo, 1997). This phenomenon has been modeled with a rat strain that sensation seeks (high responder/ HR) and that does not sensation seek (low responders/ LR). The sensation seekers or HR, both human and animal, have been found to engage in risk taking, exhibit a greater desire to exhibit novel stimuli, and are more vulnerable to drug abuse as compared to the non-sensation seekers or LR (Blanchard, Mendelsohn & Stamp, 2009; Pliner & Melo, 1997). Evidence has also been found that, during conditions of low arousal, the sensation seekers will seek out more novelty (as measured by tasting more novel foods) than the non-sensation seekers (Pliner & Melo, 1997).

With regard to the HiS and LoS rats, the HiS rats would be the sensation seekers and the LoS rats would be the non-sensation seekers. However, the biochemical evidence for the sensation seekers of the current study's model does not match the literature on the high responders and low responders (Blanchard & Mendelsohn, 2009; Dess & Minor, 1996). Understanding these rats within this model would suggest that the HiS rats are constantly seeking new arousing/stimulating stimuli, as evidenced by their propensity to abuse drugs, their greater consumption of sucrose, saccharin and sodium compared to their LoS counterparts, and their propensity to explore an open field more actively than the LoS rats, as evidenced in the literature (Carroll et al. ,2002; Carroll, Anderson & Morgan, 2007; Carroll et al., 2008; Dess & Minor,

1996; VanderWeele, Dess, 2002). It is possible that the HiS rats sensation seek because their baseline physiological arousal is lower than the optimal level that they would prefer to sustain, and that their level of optimal arousal is high overall. The literature does indicate that their baseline physiological arousal is lower than the LoS rats (Dess & Minor, 1996). The LoS rats, on the other hand, may not sensation seek because their baseline physiological arousal is already at or above the optimal levels they would prefer to sustain. The literature documents an elevated physiological arousal at baseline, and a greater emotional response in an open field experiment, that would suggest optimal or above optimal levels of arousal at baseline (Dess & Minor, 1996). These characteristics, along with their lower consumption of saccharin, sodium, and sucrose, as compared to the HiS strain suggest that the sensation seeking/optimal arousal model is a good fit as it adequately accounts for the data in the literature and succinctly explains why these behavioural differences exist between the HiS and LoS rats (Dess, 2000). Within the framework of this sensation seeking/ optimal arousal paradigm the hypotheses will now be elaborated upon.

2.10 Hypotheses Based on the Sensation Seeking/ Optimal Arousal Paradigm

It should be clarified that for simplicity purposes, the word "consumption" will be used to mean avidity scores and preference scores as well as total gram consumption. Thus if a hypothesis predicts that consumption will decrease, this means that the prediction is that preference scores, avidity scores and total gram consumption will decrease.

The first hypothesis was that during stress days versus control days both strains will decrease their consumption. However, the LoS rats will decrease their overall food intake more than the HiS rats, as supported by the previous literature (Dess & Minor, 1996). The literature has shown that restraint stress causes a decrease in consumption, and it has been theorized that

this is because coping takes precedence over eating during stress (Dess & Minor, 1996; Krebs et al., 1996; Krebs et al., 1997). When stressed, the body's main concern is to re-establish homeostasis and to keep the organism safe. This is evidenced by the sympathetic nervous system response to a stressor when less important functions such as digestion and salivation decrease and resources are re-directed to the muscles (which would be important when fleeing or attacking a threat) (Comer, 2010). Thus the nervous system response, when the rats are exposed to the stressor, should result in a similar fight or flight response where eating becomes less important than fleeing or fighting and thus consumption decreases as a result (Brunton, Russsel, & Douglas, 2008; Comer, 2010; VanderWeele,Dess & Castonguay, 2002). It was theorized that the LoS rats would decrease their consumption more than the HiS rats because, according to the sensation seeking/optimal arousal paradigm, the LoS rats are already so highly aroused that any extra stimulation is unpleasant. Thus it is likely the LoS rats will be more responsive to the stressor than the HiS rats. This will cause the LoS rats to be eating less because they are focused on coping with the stressor, whereas the HiS rats will also be coping and eating less than during control, but they are less reactive to arousal caused by the stressor; thus their consumption will not decrease as much as the consumption of the LoS rats during stress.

The next hypothesis was that during control days the LoS and HiS rats will not differ in their consumption of the citric acid flavoured food, as supported by the literature (Dess, 2000). This may be because citric acid is innately unpleasant and thus both strains, regardless of arousal level, will find the citric acid equally unpleasant due to innate taste aversions to that flavour (Miller, 2008). However, it was predicted that during the stress condition consumption of the citric acid flavoured food will decrease in both strains, but this decreased consumption will be exaggerated in the LoS strains compared to the HiS strain. Previous literature has cited a

decrease in citric acid intake during stress among non-bred strains; however, no such results exist for the HiS and LoS strains (Howell, et al., 1999). In the Howell et al. (1999) study it was proposed that stress resulted in an increased sensitivity of the normal rat population to the aversive taste of the citric acid. This increased sensitivity is what resulted in a decreased consumption of the citric acid solution during stress (Howell, et al., 1999). Therefore, in the current study it is predicted that the stress will cause an increased aversion to the citric acid flavoured food. But since LoS rats have been cited in the literature to be more reactive to an acute stressor than the HiS rats, it is predicted that the LoS rats will decrease their consumption of the citric acid flavoured food during stress more so than the HiS rats (VanderWeele, Dess, and Castonguay (2002). This is due to the greater reactivity of the LoS rats to the stressor than the HiS rats resulting in a greater increase in aversion to the citric acid flavoured food of the LoS rats compared to the HiS rats.

The third hypothesis was that during the non-stress condition, sucrose will be consumed in greater amounts by the HiS strain than the LoS strain as this has been previously demonstrated in the literature (Dess, 2000). In regard to the sensation seeking/optimal arousal model, the LoS rats may find sucrose too sweet and too arousing, and thus in an effort to modulate their arousal level, they will consume less of the sucrose flavoured food, even though sucrose is an appetitive stimulus. However, if the HiS rats on the other hand mimic sensation seekers, they will consume more of the sucrose flavoured food. With regard to consumption of sucrose during stress, there is no literature for the HiS and LoS strains. However, it has been shown that there is an increased consumption in rats of sucrose during stress because sucrose has been shown to attenuate the HPA response by decreasing plasma corticosterone levels, thus making consumption of sucrose during stress a rewarding behaviour (Christiansen et al., 2011; Dess, 1992). Hence, it was

predicted that LoS rats will show an increase in consumption of the sucrose flavoured food during stress, and HiS will show an increase in consumption of the sucrose flavoured food during stress as compared to control; however this increase in sucrose consumption will be of a lesser magnitude for the HiS rats compared to the LoS rats. If the LoS rats are always over aroused but sucrose is found to decrease the arousal of the stress response, then the LoS rats will be more motivated to consume sucrose during stress than the HiS rats in an effort to regulate their optimal arousal level. Thus both strains should show an increase in consumption of the sucrose flavoured food, but the increase should be greater for the LoS rats compared to the HiS rats.

The next hypothesis was that quinine consumption will not differ between the HiS and LoS strains during the non-stress days, as has been cited in the literature (Dess, 2000). This prediction follows a similar logic to the citric acid hypothesis. Because quinine is a bitter tasting and thus innately aversive compound, there innate, adaptive, taste aversions which cause both the HiS and LoS strains to find quinine aversive (Miller 2008). With regard to stress and its effects on quinine preference it has also been found in the literature within normal population that stress has no effect on the consumption of quinine compared to non-stress (Howell, et al., 1999). However, it was predicted within the sensation seeking/optimal arousal paradigm that the LoS rats will reduce their consumption of quinine during the stress condition as the extra stimulation from the stressor will sensitize them to the taste of quinine resulting in an exaggerated avoidance of the taste. The HiS rats, however, should respond similar to the normal population and thus their quinine consumption during stress should not change: they are not as easily affected by the stressor because their optimal level of arousal is either higher than the LoS rats or takes more stimulation to reach.

It was hypothesized that during the non-stress condition the HiS rats would consume greater amounts of the sodium chloride flavoured food compared to the LoS rats , as cited in the literature (Dess, 2000). As sensation seekers, the HiS rats should be seeking the extra stimulation which is provided by the flavoured food, whereas the LoS rats will still prefer the sodium chloride flavoured food to plain food, but as they more quickly reach/exceed their optimal level of arousal, they will stop their consumption of the sodium chloride sooner than the HiS rats. It was hypothesized that during stress the LoS rats will have a greater intake of the sodium chloride flavoured food as compared to control, and the HiS rats will also have an increased intake of the sodium chloride flavoured food; however this increase will be greater for the LoS rats. The reason for this prediction is that there is evidence in the literature of rodents increasing their sodium intake as a result of the chemical changes of the stress response (such as an increase the levels of various stress hormones) (Tarjan & Denton, 1991). If the earlier study by VanderWeele, Dess, and Castonguay (2002) was correct, and LoS rats do have higher baseline levels of plasma corticosterone and higher levels after exposure to a stressor, then it is logical to predict that the LoS rats will consume greater amounts of the sodium chloride flavoured food as a result of their higher levels of stress hormones in an effort to reach optimum arousal. If the LoS rats are indeed extra aroused all the time, and sodium consumption does alleviate the stress response, then the LoS rats should be more strongly motivated to consume sodium chloride in an attempt to lower their level of arousal in order to achieve their optimal level. The HiS rats will also be motivated to consume sodium by the same mechanism, but, because the sensation seeking/optimal arousal model suggests that their optimal level is higher than the LoS rats, less sodium is needed to reach their optimal level.

The final hypothesis was that the expected phenotypic difference of saccharin consumption will be seen for the saccharin flavoured food during the non-stress condition (Dess & Minor, 1996; Nachman, 1959). However, during the stress condition, it was hypothesized that consumption of saccharin for the LoS rats will decrease and the consumption will remain the same for the HiS rats. One of the studies regarding saccharin consumption under stress for HiS and LoS rats did not show significant results; however it did approach significance, and if it had been reached, the results would have been as the present study predicts (Dess & Minor, 1996). It has also been shown within the regular population that there is a decrease in saccharin consumption (Dess, 1992). Within the framework of the sensation seeking/optimal arousal model, the LoS rats will be over aroused again by the stressor; however the literature suggests that the LoS rats will increase their saccharin consumption. This may be because saccharin holds some benefit or placating effect during stress which may cause the LoS rats to increase their consumption in an effort to cope with the stressor. This may be similar to how sucrose consumption is often increased during stress (Christiansen et al., 2011; Dess, 1992). Again, any change in consumption patterns results from an effort to maintain their optimal level of arousal.

2.11 A Possible Effect of Calories and Texture on Consumption

Despite the fact that these predictions are rooted in the literature, the conditions of the current study differ from the previous studies concerning the taste preferences of the HiS and LoS lines. Namely, the previous studies used liquids with no calories, whereas the current study used solid food with calories (Dess, 2000). Because of the addition of calories in the present study there is the possibility that the calories may change the reinforcing qualities of the flavoured foods.

The predictions for taste preferences were made based upon the flavours of the different foods. For example, the hedonic tastes such as sweet and salty were predicted to be preferred more than the anhedonic tastes such as sour and bitter. The hedonic tastes are preferred because sweet and salty foods signal the presence of nutrients, calories and electrolytes respectively. Sour and bitter on the other hand signal that a food may be poisonous or has spoiled, and thus are normally avoided (Pinel, 2009). However, calories are reinforcing on their own and thus the presence of calories in the test foods of the current study could override the anhedonic tastes, and could possibly override the differences in taste preferences seen between the HiS and LoS rats.

Similarly, changing the products from flavoured liquids to flavoured solids can change the outcome as well. The literature has shown that liquids are consumed in greater amounts compared to solids, even when the qualities of each are equal (de Wijk et al., 2008; Hogenkamp et al., 2012; Zijlstra et al., 2008). This is due in part to the fact that liquids can be consumed at a quicker rate (less eating effort is required) which allows more to be consumed before satiety signals can be interpreted by the PGC, thus ending the meal. Because liquids are consumed in greater amounts than solids, it is possible for a ceiling effect to occur with the solid food before differences in consumption between the two lines is apparent.

For example, large volumes of liquid were consumed by the subjects in the Dess (2000) study which investigated the taste preferences of the HiS and LoS lines. Across the experiments for each of the different flavours, each rat consumed an average of 459.38 g (or milliliters) of liquid for the duration of each experiment. As each experiment had seven 24 hour two-bottle preference tests, this works out to an average of 65 g (or milliliters) of the flavoured solution consumed daily. Compared to the average food consumption of full grown rats at 15.0 to 20,0 grams per day and the average volume of a rat's stomach being 3.4 mL, it is clear that these

liquids are passed through the gastrointestinal system much quicker than solid foods. Therefore fluids can be consumed in much greater amounts than solid foods, and thus it is possible that a ceiling effect was reached (McConnell, Basit, & Murdan, 2008; Nebendahl, 2000).

The changing of the test solutions from liquids with no calories to solids with calories was done in the interest of creating circumstances that are more ecologically valid than the previous studies. Animals and humans traditionally acquire nutrients and calories through the consumption of solid foods: therefore using solid foods in the present study is a more accurate analogue of human eating behaviour. However, the possible effects just outlined with changing the caloric content and texture of the test solutions should be kept in mind.

2.12 Literature Review Summary

The literature just reviewed has shown that the results of stress and eating research, with the normal rat population, are conflicting. Some studies have shown an increase and others a decrease in consumption, depending upon the particular experimental conditions (Krebs et al., 1996; Macht et al., 2001). With regard to the HiS and LoS strains of rats, not only have the strains been shown to differ in consumption of saccharin, but also in their propensity for drug use and their stress responsiveness; they have also been shown to exhibit some differences in other flavour preferences (sucrose, sodium chloride, etc.) as well as changing their eating habits differentially when under stress (Carroll, Anderson & Morgan, 2007; Dess, 2000; Dess & Minor, 1996; VanderWeele, Dess, & Castonguay, 2002). The LoS rats have been shown to decrease consumption during stress and the HiS rats have been shown to keep their consumption relatively the same (Dess & Minor, 1996). These differences between the strains have been assumed to be due to genetic differences which are a result of selective breeding. This may show possible

genetic linkage between these behavioural traits, making these strains of rats useful in the research of emotionality/stress and eating.

The sensation seeking model was presented as a potential theoretical framework within which the characteristics/behaviours of the HiS and LoS strains may be understood. If this framework is found to explain the behaviour of the HiS and LoS strains, this may allow previous studies using the HiS and LoS strains to be framed within the sensation seeking model as well. This may then lead to new insights regarding why the HiS and LoS strains behave as they do and insights regarding the behaviour/propensity for certain behaviours of humans who are considered to be sensation seekers or non-sensation seekers. If the results are not found to support the predictions of the sensation seeking/optimal arousal paradigm, this may suggest that alliesthesia is occurring and that the addition of calories to the test solutions and the use of solids instead of liquids had an effect on the taste preferences of the HiS and LoS strains. The literature has shown calories and the texture of foods to influence consumption and preference patterns, and thus should be considered when interpreting the results of the current study (Laeng et al., 1993; Berridge, 1991; Griffioen et al., 2012; Holman, 1969; Moskowitz et al., 1976; Rolls et al., 1988). As was reviewed in the literature, the consumption of nutrients and calories may affect the taste preferences of the subjects. And thus, this should be considered when interpreting the results of the study (Laeng et al., 1993; Berridge, 1991; Griffioen et al., 2012; Holman, 1969; Moskowitz et al., 1976; Rolls et al., 1988).

With this previous literature in mind, the purpose of the present study is to further examine how stress affects consumption in the HiS and LoS strains, and the differences in flavour preferences between the strains (Dess, 2000; Dess & Minor, 1996). The goal of this research is to determine how stress can alter these heritable preferences. This was explored by

submitting the HiS and LoS strains to a restraint stressor and observing the resultant changes in consumption between the two strains. Additionally, the present study attempted to replicate previous taste preference findings of the HiS and LoS strains with flavoured foods instead of flavoured liquids (Dess, 2000). It is predicted that the results will fall within the sensation seeking/optimal arousal framework. For example, the HiS rats are expected to have greater preference scores for all hedonic tastes (sweet, salty, saccharin) compared to the LoS rats and no differences are expected between the lines for the anhedonic tastes (sour and bitter). With regard to the effects of stress, it is predicted that stress will decrease consumption but this decrease is expected to be greater for the LoS rats compared to the HiS rats because the LoS rats as sensation non-seekers are more sensitive to the effects of the stressor.

With the background literature, rationale, and goal of the current study fully explored, the methodology will now be elaborated upon.

3 Methods

3.1 Subjects

This study began with sixteen female rats supplied by the University of Minnesota, eight saccharin preferring rats (HiS) and eight saccharin non-preferring rats (LoS). The rats were experimentally naïve and approximately a year old at the beginning of the experiment. By the end of the study only fourteen rats were used to collect data. Due to poor health, two of the rats needed to be euthanized (one HiS rat and one LoS rat).

The HiS and LoS rats are outbred strains, meaning that the population consists of animals where no mating occurs between animals that are closely related (Carroll et al., 2008). To maintain outbred status of the lines, sibling, half-sibling, and first cousin mating was avoided; additionally, every four to six generations, rats were purchased from the founding stock (i.e., Sprague-Dawley) and tested for saccharin preference. Male and female rats expressing high or low saccharin preferences were then mated with the selectively bred HiS and LoS lines, respectively (Carroll et al., 2008).

The HiS and LoS strains were initially bred for by Nachman (1959). He had initially discovered that among his sample population of rats some preferred a saccharin solution more than other rats. Thus he decided to selectively breed his sample rats to produce two stains, one that was selected for saccharin preference over water, and the other that was selected to prefer water over a saccharin solution. Later on, this line was further investigated by Dess and Minor (1996) who noticed within their population of Holtzman Sprague Dawley rats that there was a male rat that did not seem to prefer saccharin to water, which was unusual for this strain. They thus decided to breed this rat with an average consuming female to create the LoS line. To create

the HiS line, a high preferring male rat was bred with an average consuming female rat (Carroll et al., 2008; Dess & Minor, 1996). From the offspring, the extreme scoring rats were bred together (the high with the high and the low with the low). The extreme scoring rats were those among that generation of offspring with the highest and the lowest preference scores (saccharin intake (ml)/ total fluid intake (ml)) during a two bottle preference test. Through this selective breeding process, the phenotypic differences of the HiS and LoS rats emerged by the third generation.

It should be noted that each of the rats supplied by the Minnesota lab was tested by the Charles River Research Animal Diagnostic Services for various bacteria and diseases. The rats in the current study tested positive for the bacteria Helicobacter Genus and Campylobacter Genus. As a result of this, the animals were required to remain in quarantine for the entirety of the experiment. This was to ensure the health of the other animals housed in the animal care facility. Because the animals tested positive for those bacteria, the results for this study will now have to be interpreted with caution as some effects of the bacteria could affect consumption and responses to stimuli (Charles River Laboratories International Inc., 2011; Colorado State University, 2009). Each bacterium and its possible effects will now be described.

It is common for mammals to have at least one Helicobacter Genus species in its system, and Helicobacter Genus has been found in both wild and laboratory rats. This bacterium is transmitted via the consumption of contaminated fecal matter and may also be transmitted in the air, as dust and other particulate may move from cage to cage. Helicobacter Genus has been found to colonize the cecum, colon, gall bladder, liver, and less commonly the stomach. An infection can result in rectal prolapse, diarrhea and possibly cancer of the liver or colon; however, most carriers do not exhibit any symptoms. A positive result for Helicobacter Genus

does not specify where the bacteria have colonized. If the bacterium is colonized to the gut or liver, the subjects' response to various stimuli (such as food consumption) may be affected. Thus, any conclusions made using these particular subjects must be interpreted with caution, as the presence of Helicobacter Genus could interfere with the effect of the stimuli on the animals' behaviour (Charles River Laboratories International Inc., 2011).

Campylobacter Genus, the other bacterium found in the subjects system, is less common than Helicobacter and has been shown to result in a mild to severe infection of the gastrointestinal system. Campylobacter Genus is also transmitted through the consumption of contaminated feces. Some symptoms of this infection include watery/ bloody diarrhea, fever, abdominal cramps, and nausea. Many carriers, as with Helicobacter Genus, are asymptomatic. Because this bacterium is present in these subjects, any results from this study must be interpreted with caution. If the animals were symptomatic at the time of the experiment, any of the symptoms listed above could influence the consumption patterns of the subjects, thus interfering with the results (Colorado State University, 2009).

Although it was unfortunate that the animals tested positive for these bacteria, it was still possible for the experiment to be carried out. The animals were asymptomatic during data collection as evidenced by no observed changes in consumption and behavioural patterns, or in the appearance of fecal boli. The health status and behaviours of the subjects was closely monitored throughout the study to ensure any changes in would be detected. Therefore, although one must be mindful of the bacteria when interpreting the results, the presence of Campylobacter and Helicobacter does not negate the findings and information gathered from this experiment.

3.2 Materials

3.2.1 Housing Conditions

The animals were housed at the Laurentian University Animal Care Laboratory where the temperature was kept within a range of 21-22°C. Because of the bacteria present in the subjects' system, the study was performed entirely in the quarantine room of the animal care lab. The housing room was controlled on a 12/12 hour light/dark cycle, with the lights turning on every day at 8:00 am and off at 8:00 pm. The rats were housed individually for all phases of the experiment to enable monitoring of individual food consumption.

Clear poly-carbonate cages with matching lids were used to house the animals. These cages were stored in a specialized shelving unit equipped with an air filtering system for the cages. Each rat's cage contained corn cob bedding as nesting material and a red semi-transparent tube for environmental enrichment. Teklad Rodent Diet rat food was made available through a food dispenser on the roof of the home cages, and water was made available in 500 ml plastic bottles hung from the top of the cage. The home cages were modified to hold two 500 ml plastic bottles for the saccharin preference test phase of the study.

The same clear poly-carbonate cages were used for the testing phase of the experiment; however they did not contain any bedding or environmental enrichment. For the testing phase the cages contained two 100 g ceramic dishes placed at the front of the cage and a line made with duct tape dividing the cage in half. The cage was divided to help determine which cup any food on the bottom of the cage was from to help strengthen measurement accuracy.

3.2.2 Stressor

The consumption of the subjects was monitored both during and in the absence of stress. Of interest to this study was the effect of stress on food consumption and taste preference. During a stress day the rats were exposed to restraint stress, which was applied by placing the animals in small plastic tubes. On each stress day the rats were stressed for 30 minutes and then given access to their food. The stress tubes were clear, closed at one end and had a moveable closure at the other end with a hole in it for the subjects' tails to go through. This moveable closure could be adjusted to secure any size of rat. Restraint stress has been shown to result in no injury to the animal and is an effective method of stressing rats (Krebs et al. 1996).

As previously stated, in a study by Christiansen et al. (2011), restraint stress has been shown to effectively increase plasma corticosterone levels, which has been cited as biological evidence that the animal is under stress. Thus restraint stress has been shown to be an effective stressor. The literature also indicates that restraint stress can effectively decrease consumption of food amount as well as differentially influence consumption of various flavours. In a study by Howell, et al. (1999), restraint stress resulted in decreased consumption of saccharin fluid, sodium chloride fluid, and citric acid fluid. Restraint stress did not, however, affect consumption of quinine and it was not consistent in its effects on consumption of the sodium chloride solution. Thus restraint stress was an appropriate choice for this study as changes in food preferences and total consumption are being investigated.

3.2.3 Test flavours and presentation

Five different mixtures of flavoured rat chow were used in this experiment to test the animals taste preferences. Rat chow was flavoured to test taste preferences rather than using different foods in order to keep texture and calories constant across testing conditions. These mixtures included a bitter sweet mixture (0.2% saccharin solution); a sweet mixture (2.0% sucrose solution); a bitter mixture (0.004% quinine hydrochloride solution); a salty mixture (0.90% sodium chloride solution); and a sour mixture (0.2% citric acid solution). The different concentrations were chosen based upon previous literature involving this line of rats. The exception to this was for the bitter, the sour, and the bitter sweet mixtures which were doubled from the original concentration in the literature to ensure that the flavourant could be tasted among the flavour of the rat food (Dess, 2000). The literature indicates that preference scores were the highest for the preferred flavours (saccharin, sucrose, and sodium chloride), and the lowest for the aversive flavours (quinine hydrochloride and citric acid) at the concentrations chosen for the proposed study. These particular mixtures were chosen for the proposed study to elicit the maximum behavioural response from the subjects, which would be very high preference scores for the hedonic tastes (sweet, salty, saccharin), and very low preference scores for the anhedonic tastes (bitter, sour).

These mixtures were created by taking powdered Teklad Rodent Diet, and mixing it with flavoured water of the appropriate concentration (solute (g)/ water (ml) x100). The powdered food was created by crushing the food pellets with a hammer and proceeding to grind the crushed food bits with a coffee grinder. The flavoured liquid was created by mixing powdered flavourant (sucrose, citric acid, saccharin, sodium chloride and quinine) into the water to create the desired concentration. The flavoured food mixtures were created by combining these two components.

The plain food mixture was created the same way except the powdered food was mixed with plain water.

These different flavoured food mixtures were presented to the animals in ceramic cups which hold approximately 100 g of powdered food. These ceramic cups were used during the flavour exposure period as well as during the regular testing period. The cups were secured to the bottom of the cage using hook and loop dots in order to prevent the animals from knocking over the cups. This precaution helped to ensure accurate measurements of intake.

3.3 Procedure

3.3.1 Quarantine

At the beginning of the study the rats were kept in quarantine for 3 weeks to ensure that no foreign pathogens were being introduced to the animal care facility. Saliva swabs and fecal samples were taken and sent for analysis to determine if the animals could leave quarantine. During the quarantine period the rats were handled daily by the experimenter at 9:00 am every morning and on the first day of quarantine the rats' tails were marked to aid in identification of the animals. Handling during this period was done to habituate the animals to handling by the experimenter to reduce the amount stress produced by handling during the testing phase of the experiment. The fecal samples and saliva swabs came back indicating that the subjects were positive for Campylobacter Genus and Helicobacter Genus, as was mentioned earlier. Thus the experiment was carried out in the quarantine room to ensure the cleanliness of the general population environment in the laboratory.

3.3.2 Food Habituation

Next the animals were presented with each of the food solutions (plain, sweet, sour, salty, bitter, and saccharin) overnight to ensure that there was no confounding effect of novelty when the animals were presented the solutions during the testing phase of the experiment. During this stage of the experiment the rats were presented with the individual flavour mixtures at 4:00 pm overnight and then the intake was measured at 9:00 am the following morning. Each of the flavours was presented to the animals three times over the duration of 18 days. Presentation order was counterbalanced between the different animals.

3.3.3 Test Cage Habituation

After exposure to the food flavours the rats were then habituated to the fully equipped test cages for a week. The test cages contained the two ceramic cups each with the plain food mixture in them as well as the line dividing the cage in half. The night before habituation the animals were food deprived from 16:00 until 9:00 the following morning when the rats were placed in the test cages for 30 minutes. After the 30 minute exposure the rats were placed back into their home cages. The purpose of habituation to the testing cages was to ensure that the rats had habituated to the test cage environment, reducing the arousal due to its novelty when the testing took place.

3.3.4 Baseline water intake and saccharin preference

After the habituation period, the rats had their baseline water intake measured. This was done by monitoring water intake (mL) for 24 hours while still having access to food in their home cages. Fluids were presented to the animals in the home cage in two 500 ml plastic bottles.

During this baseline measurement the water levels were checked and filled when required at 16:00 and 20:00. The baseline water measurement was used as a comparison to the saccharin consumption in order to validate the rat strains (His and LoS).

Once the baseline water intake of the rats was determined, a two-bottle preference test with saccharin (0.10%) and water was performed with each solution presented in one of the 500 mL bottles. Over 24 hours the rats had access to both water and a saccharin solution, and the fluid levels of both solutions were checked at 4:00 pm and 8:00 pm. After this 24 hour period, the consumption of each of these solutions (mL) was measured. During this period the rats were given ad libitum access to their food.

The preference scores were calculated as the amount of saccharin solution consumed divided by the total amount of fluids consumed over the 24 hour period. The preference scores were used to confirm the strain (HiS or LoS) of the subjects. As mentioned earlier, a preference score significantly above or below 0.50 indicates preference or non preference respectively (Dess & Minor, 1996). This particular method of measuring preference score was used in a study by Dess and Minor (1996) and thus was replicated for this study.

3.3.5 Testing phase

Once the strains were confirmed, they were tested on their food preferences both during and in the absence of stress. Testing occurred over 40 days which included one rest day in between each testing day. Testing continued until all rats had been exposed to each flavour (sweet, sour, salty, bitter, saccharin) four times. Of the four presentations of each flavour, two presentations were on a stress day and the other two presentations were on a control day. The order in which the food flavours were presented to the rats was counter-balanced between

subjects. The counter- balancing of stress and control days can be seen in Appendix A and the presentation order of the flavours can be seen in Appendix B.

The food preference tests began by food-depriving the rats for 17 hours from 4:00 pm until testing began at 9:00 am the following morning. During testing, the rats were presented with one of the five flavoured food mixtures in a cup. This cup was adjacent to another cup containing the plain food mixture. The subjects were given a choice of plain or flavoured food so that preference scores (flavoured food consumed (g) ÷ total food consumed (g)) could be measured for the different flavoured mixtures. Presentation of the two food cups occurred over a period of 30 minutes. The positioning of the flavoured and plain food cups was counterbalanced between rats.

On stress days, during testing, the animals being stressed were placed into the restraint stress apparatus for 30 minutes, during which time the control animals remained in the test cages without access to the foods. After 30 minutes the stress animals were removed from the restraint apparatus and placed in the test cage with the two cups of food present (one flavoured, one not flavoured). As one stressed animal was placed into the test cage, one control animal was given access to the foods and this continued until all animals were in the test cage with access to the food mixtures. The stress and control days were alternated among the animals with half being stressed one day and half being stressed the next test day (the remaining half on each stress day was the non-stress group for that day). Of the subjects being stressed or not stressed on any given test day, half of them were HiS rats and the other half were LoS rats. For the next 30 minutes while both the stress and non-stress animals were given access to the food cups, behavioural observations were taken of each animal. These behaviours will be further described, alongside the other measures of this experiment, later in the document. At each 30-second mark the

behaviour being exhibited by one of the animals was noted. Then after another 30-second interval the behaviour of the adjacent animals was observed. This continued until the end of the testing session.

For the duration of the procedures just described, various measures were taken for the purposes of investigating the hypotheses, as well as for monitoring the health of the animals. These measures included the weight of the rats, consumption of the food mixtures, liquid consumption (water and saccharin solution), avidity score of both food and saccharin, preference scores and behavioural observations. Each measure will now be described in greater detail.

3.3.6 Weights

For the duration of the study, beginning during the food habituation portion of the experiment, the animal's body weight was measured daily in grams to one decimal place using a 3 balance beam scale. Their body weight was measured in order to monitor the health status of the animals. This was done to ensure that the animals were not losing excessive weight due to the food restriction portions of the study. Body weight measures were also used when calculating avidity scores.

3.3.7 Consumption (grams)

During habituation to the food mixtures as well as during the flavour preference tests of the experiment, consumption of the powdered food was measured in grams to one decimal place using an electronic digital scale. These values were used to calculate other scores such as preference and avidity.

3.3.8 Consumption (milliliters)

During the saccharin preference and baseline water intake phase, the amount of fluid consumed was measured in milliliters using graduated cylinders. Fluid consumption was measured to one decimal place. These values were used to calculate other scores such as preference and avidity.

3.3.9 Avidity

Avidity is a measure which calculates intake as a percentage of the animals' body weight, as well as accounting for changes in consumption due to the presence of saccharin (Dess and Minor, 1996). This calculation helps to control for any differences in weight among the subjects. Avidity is calculated by taking total intake during the testing session and subtracting the baseline intake (if this difference value is 0 this suggests that no additional food was eaten when a saccharin solution was present as compared to during baseline). This difference is then divided by the body weight of the animal and multiplied by 100 to make the score a percentage of the rats' body weight (Dess & Minor, 1996). For example, imagine a HiS rat with a body weight of 319.2 g and a baseline water intake of 58ml. With an avidity score of 17.66, the HiS rat would have consumed a total of 114.4 ml when both water and saccharin were present ([114.4 ml – 58 ml] ÷ 319.2 x100). By comparison, a LoS rat with an avidity score of 4, but with the same baseline water intake and body weight, would have consumed a total of 70.77 ml when both water and saccharin were present ([70.77 ml- 58 ml] ÷ 319.2 x100). This clearly demonstrates the consumption differences between the HiS and LoS lines.

For avidity scores of food, the mean consumption of the plain food mixture during the habituation phase of the experiment was the baseline measurement. For avidity scores of

saccharin, the value from the 24-hour-water-only baseline day was used to calculate the avidity score of saccharin.

3.3.10 Preference score

A preference score was calculated by taking target solution consumption and dividing it by total intake during the testing days. For food consumption the flavoured food consumption (grams) was divided by the total intake during that testing day (grams). For saccharin consumption during the two-bottle preference test, consumption of the saccharin was taken and divided by the total fluid intake during that 24 hour two-bottle preference test period (Dess & Minor, 1996).

3.3.11 Behavioural observation

During the testing phase when the animals' food preferences were being examined, behaviours were monitored while the animals had access to the two food cups. The behaviours accounted for included rearing, licking, scratching, sniffing, being mobile/walking, grooming, being frozen/doing nothing, biting anything, and eating. The purpose of monitoring these particular behaviours was to determine if and when the animals were experiencing stress. Changes in amount of movement (increase or decrease), increased grooming, exploration, and increased walking or being frozen, have been cited as behavioural evidence that an animal is experiencing stress (CCAC, 2003).

4 Results

4.1 Analyses

All analyses were performed using IBM SPSS Statistics 19 software. A preliminary 2 x 5 x 2 x 2 mixed factorial ANOVA was performed with trial as a variable with two levels (i.e. first and second presentation of each flavour during each stress condition). Trial was not found to have a main effect or interact with the other variables so two 2 x 5 x 2 mixed factorial ANOVAs were used for the final analyses. For both of these ANOVAs the three independent variables were stress, tastes, and phenotype. Stress was a within subjects variable with two levels. These levels include the control condition (no stress) where the rats were placed in the test cages without having been exposed to the stressor and the stress condition where the rats were exposed to the restraint tubes before being placed in the test cages. The independent variable tastes was a within subjects variable as well but with five levels, one for each flavour. These levels include the flavours sweet (2.0% sucrose), bitter-sweet (0.2% saccharin), bitter (0.004% quinine), sour (0.2% citric acid), and salty (0.90% sodium chloride). Phenotype was a between subjects variable with two levels. These two levels were the HiS strain and the LoS strain of rats. Both ANOVAs were two-tailed because there was no previous literature upon which to predict the direction of any of the effects. Specifically, no previous literature with these lines exists examining the effects of stress on the taste preferences of these lines for solid caloric foods of different flavours.

Each of the ANOVAs used a different dependent variable for the analysis. The first ANOVA used the dependent variable preference scores for each flavour of food as measured

during testing. The second ANOVA used the dependent variable avidity scores for each flavour of food as measured during testing.

Along with the two ANOVAs, three sets of independent samples t-tests were also performed. One t-test was performed using preference scores to confirm the HiS and LoS strains. This t-test was one-tailed in an effort to increase the power of the test and to accommodate for the smaller sample size used in the current study. A one-tailed test is justified because the difference in saccharin consumption between the lines is so heavily supported by previous literature (Dess, 2000; Dess and Minor, 1996; Nachman, 1959) Another t-test was performed using average weight (g) to determine if either of the strains was heavier than the other. This was a two-tailed test because there was no theoretical basis upon which to predict the direction of this effect. The last t-tests compared the occurrence of different behaviours during stress and during control. This comparison was one- tailed, as supported by the literature (CCAC, 2003).

4.2 Assumptions

When performing an ANOVA, certain assumptions must be met. For the analyses at hand the assumptions of normality (as determined by standard skewness and kurtosis values below 3) and homogeneity of variance (as determined by non significant Levene's Test of Equality of Error Variances results) were both met for all of the dependent variables and for both ANOVAs respectively. Thus using an ANOVA to analyze this data is appropriate.

For the ANOVA that used preference scores, the assumption of sphericity (as determined by Mauchly's Sphericity) was met. However, for the ANOVA that used avidity scores, the assumption of sphericity was violated for the tastes x stress interaction. To correct for this

violation, the Greenhouse Geiser correction will be reported when reporting the *F* value and the degrees of freedom for this result.

4.3 Confirming characteristics of the phenotypes

The HiS and LoS strains are identified based upon their differing consumption of an aqueous saccharin solution. The HiS rats have been found to have greater preference scores for the saccharin solution than the LoS rats (Dess & Minor, 1996; Nachman, 1959). Figure 1 shows that the HiS and LoS rats had preference scores of 0.81 (SE = 0.03) and 0.71 (SE =0.05), respectively. These preference score values are comparable to the values stated for each strain in previous literature using the HiS and LoS rats (Dess & Minor, 1996). Although the mean preference score for the HiS rats was higher than that for the LoS rats, a one-tailed independent samples t- test revealed that this difference approached, but did not attain, significance (t (12) = 1.67, p= .06). It is likely that this result fell short of significance due to the small sample size of each strain (n = 7). A smaller sample size was used because these rats are a specialized breed and therefore difficult to obtain. In addition, the sample size in the current study was reduced due to health issues. For the purpose of this thesis, it is proposed that the study will proceed based on the assumption that the difference in preference scores between the HiS and LoS observed in the current study is real.

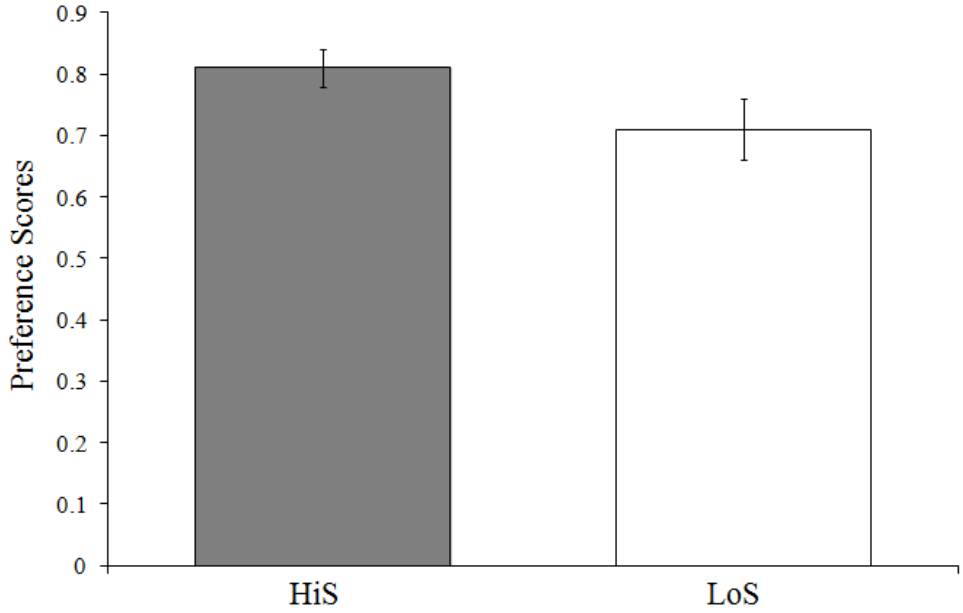

Figure 1: Preference scores of the HiS and LoS strains

The two strains were also found to differ significantly in body weight (t (12) = -2.49, p<.05). The LoS rats (M=384.54, SE=15.45) were found to be heavier than the HiS rats (M=337.24, SE=11.04). This is not an uncommon finding in the literature, especially among the female rats (Dess & Minor, 1996). A listing of the recorded weights of the HiS and LoS rats can be viewed in Appendix C. In order to account for weight as a potential confound avidity scores have been used in the previous literature, and were used in the present study, to account for differences in body weight among the animals (Dess, 2000). Avidity scores are a measure of intake as a percentage of the rats' body weight.

4.4 Line differences in flavour consumption

It was hypothesized that the taste by phenotype interaction, for both avidity and preference scores, would be significant. Specifically it was predicted that the HiS strain would consume significantly greater amounts of the sweet, salty, and saccharin mixtures as compared to

the LoS rats. It was also predicted that the consumption of the sour and bitter mixtures would not differ between the two strains. When using avidity scores, the results did not support these hypotheses. The taste by phenotype interaction was found to not be significant (F (4, 9) = 0.15, p>.05). Thus the consumption of the different tastes was not dependent upon the phenotype of the rats. The mean avidity score of the sweet mixture for the HiS rats was 1.82 (*SD*= 0.52) and was 2.42 (*SD*= 0.52) for the LoS rats. The mean avidity score of the sour mixture for the HiS rats was 1.91 (*SD*= 0.53) and was 1.69 (*SD*= 0.53) for the LoS rats. The mean avidity score of the salty mixture for the HiS rats was 1.87 (*SD*= 0.46) and was 1.85 (*SD*= 0.46) for the LoS rats. The mean avidity score of the bitter mixture for the HiS rats was 1.79 (*SD*= 0.38) and was 1.61 (*SD*= 0.38) for the LoS rats, and finally the mean avidity score of the saccharin mixture for the HiS rats was 1.72 (*SD*= 0.44) and was 1.83 (*SD*= 0.44) for the LoS rats.

When using preference scores, the results tentatively support the hypothesis. The taste by phenotype interaction approached significance (F (4, 9) = 2.52, p=.053, η^2=.174). This result was treated as significant, and thus an analysis of simple effects was performed. The simple effects tests revealed that the LoS rats consumed significantly greater amounts of the sweet solution as compared to the HiS rats (t= -2.50, p<.05), which is a significant result, but in the opposite direction than predicted. The mean preference score of the sweet mixture for the HiS rats was 0.43 (*SD*= 0.05) and was 0.59 (*SD*= 0.05) for the LoS rats, the mean preference score of the sour mixture for the HiS rats was 0.42 (*SD*= 0.07) and was 0.50 (*SD*= 0.07) for the LoS rats, the mean preference score of the salty mixture for the HiS rats was 0.43 (*SD*= 0.06) and was 0.58 (*SD*= 0.06) for the LoS rats, the mean preference score of the bitter mixture for the HiS rats was 0.50 (*SD*= 0.05) and was 0.49 (*SD*= 0.05) for the LoS rats, and finally the mean preference score of

the saccharin mixture for the HiS rats was 0.47 (*SD*= 0.05) and was 0.50 (*SD*= 0.05) for the LoS rats. These findings are presented in Figure 2.

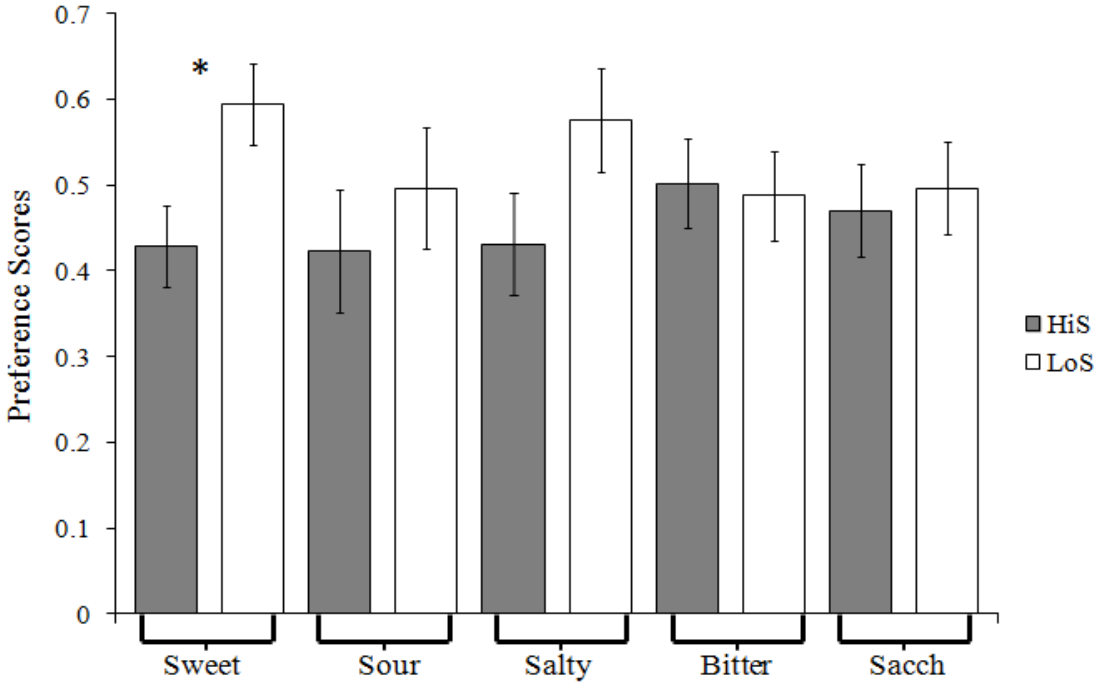

Figure 2: Mean Preference scores per Flavour by Phenotype. Consumption of the sweet mixture by LoS rats was significantly greater than consumption of the sweet mixture by HiS rats (*t*= -2.50, p< .05)

4.5 Main effect of stress on overall consumption

It was also hypothesized that there would be a main effect of stress on overall consumption (both avidity and preference scores). Specifically, it was predicted that consumption would decrease during stress days as compared to control days. When using preference scores the results did not support this hypothesis. No significant difference was found between preference scores on stress days as compared to preference scores on control days (F (1, 12) = 1.70, p>.05). The mean preference score during stress was 0.48 (*SD*=0.03) and the mean preference score during control was 0.50 (*SD*=0.04).

When using avidity scores the results supported the hypothesis. The main effect of stress on avidity scores was found to be significant (F (1, 12) = 19.76, p<.05, η^2=.622). It was found that consumption during stress days was significantly less than consumption during control days. The mean avidity score during stress was 1.42 (SD= 0.28) and the mean avidity score during control was 2.28 (SD= 0.26). These findings are presented in Figure 3.

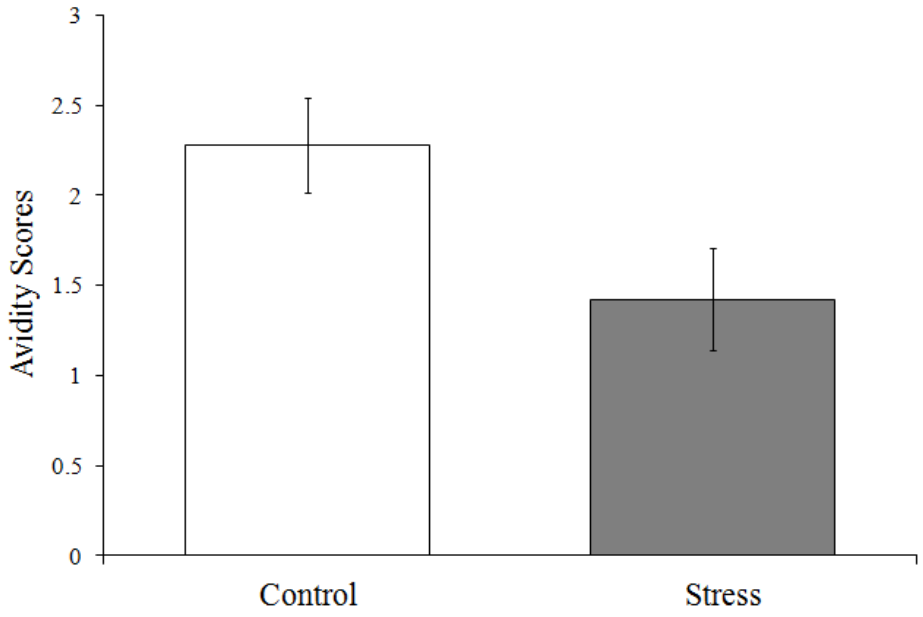

Figure 3: Mean Avidity scores for Control and Stress day

4.6 Differential consumption of flavours during stress

It was also predicted that consumption of the different flavours would vary depending upon stress condition. Specifically it was predicted that the consumption of hedonic tastes would increase during stress compared to control and the consumption of anhedonic tastes would remain the same or decrease during stress compared to control. The hedonic tastes include the

sweet, salty and saccharin flavoured mixtures and the anhedonic tastes include the bitter and sour flavoured mixtures.

When using avidity scores the data did not support the hypothesis pertaining to the anhedonic tastes, the consumption of the bitter and sour mixtures did not differ during the stress condition compared to the control condition, as predicted. The hypothesis was also not supported pertaining to the hedonic tastes; there was no change in consumption of any of the hedonic tastes during the stress condition as compared to the control condition. Therefore there was no difference in avidity scores as a function of stress. The taste by stress interaction was found to not be significant (F (4, 24.51) = 0.59, p>.05). The mean avidity score for the sweet mixture in the absence of stress was 2.27 (SD= 0.23), and was 1.96 (SD= 0.78) during stress. The mean avidity score for the sour mixture in the absence of stress was 2.11 (SD= 0.38) and was 1.49 (SD= 0.42) during stress. The mean avidity score for the salty mixture in the absence of stress was 2.39 (SD= 0.28) and was 1.32 (SD= 0.42) during stress. The mean avidity score for the bitter mixture in the absence of stress was 2.34 (SD= 0.37) and was 1.06 (SD= 0.29) during stress, and the mean avidity score for the saccharin mixture in the absence of stress was 2.27 (SD= 0.35) and was 1.28 (SD= 0.35) during stress.

Although the interaction was not found to be significant, a paired samples t-test comparing consumption during stress and consumption during control of each flavour revealed some significant differences. This post-hoc was performed on a non-significant result because upon visual inspection of the graphed data there is a large amount of distance between the control and stress day means for each flavour; thus the post-hoc was performed to see if these distances were significant. Consumption of the sour (t (13) = 2.14, p<.05) salty (t (13) = 3.77,

p<.05), bitter (*t* (13) = 3.31, *p*<.05) and saccharin (*t* (13) = 3.07, *p*<.05) mixtures decreased significantly during stress as compared to control. These results can be seen in Figure 4.

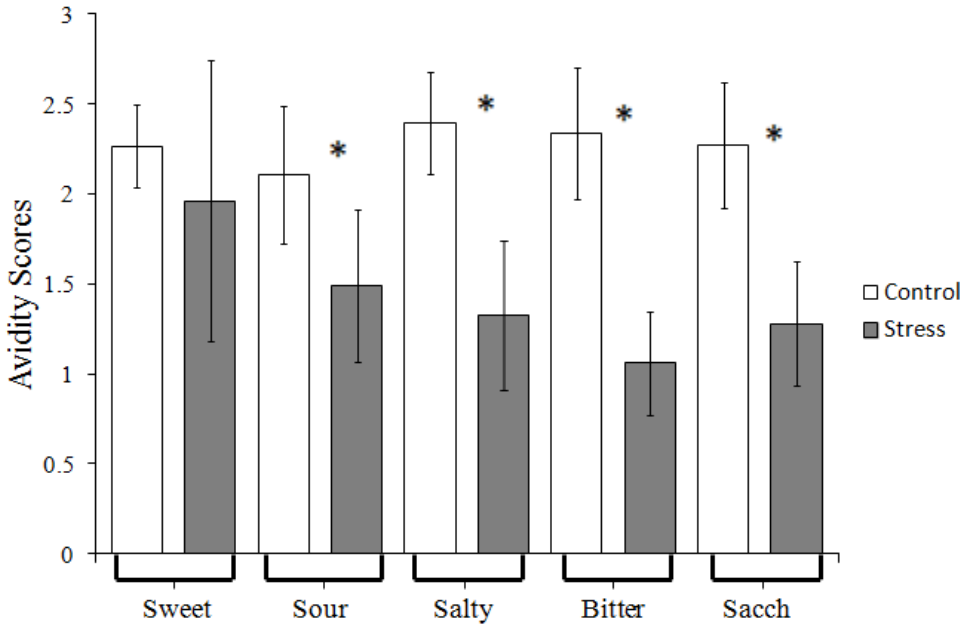

Figure 4: Mean Avidity scores per Flavour during Stress and Control days. Consumption of sour (*t*=2.2, *p*<.05), salty (*t*=3.63, *p*<.05), bitter (*t*=3.20, *p*<.05), and saccharin (*t*=3.02, *p*<.05) solutions was significantly less during stress compared to control when post-hoc paired samples t-tests were performed.

The same conclusions that were drawn from the previously discussed avidity score data can be drawn from the preference score data. The hypothesis was not supported for the hedonic or anhedonic tastes. The taste by stress interaction was found to not be significant (*F* (4, 48) = 0.811, *p*> .05). The mean preference score for the sweet mixture in the absence of stress was 0.57 (*SD*= 0.05), and was 0.45 (*SD*= 0.05) during stress, the mean preference score for the sour mixture in the absence of stress was 0.46 (*SD*= 0.06) and was 0.46 (*SD*=0.06) during stress, the mean preference score for the salty mixture in the absence of stress was 0.48 (*SD*= 0.07) and was 0.53 (*SD*= 0.05) during stress, the mean preference score for the bitter mixture in the absence of stress was 0.54 (*SD*= 0.06) and was 0.45(*SD*= 0.04) during stress, and the mean

preference score for the saccharin mixture in the absence of stress was 0.46 (*SD*= 0.05) and was 0.51 (*SD*=0.07) during stress.

4.7 Effect of stress on flavour consumption of LoS rats compared to HiS rats

The final hypothesis of this study was that any of the effects of stress on preference scores and/or avidity scores would be exaggerated in the LoS population as compared to the HiS population. For example, if stress were to result in an increased consumption of hedonic tastes and a decreased consumption of anhedonic tastes, the HiS and LoS rats would exhibit these changes but this effect would be greater for the LoS rats. This hypothesis was examined through the three way interaction of taste by stress by phenotype.

When using avidity scores, the hypothesis was not supported. The three way interaction of taste by stress by phenotype was found to not be significant (F (4, 9) = 0.64, p> .05).The avidity scores for the HiS rats were as follows. On control days the mean avidity scores were 2.46 (*SD*= 0.32), 2.02 (*SD*= 0.54), 2.38 (*SD*= 0.40), 2.51 (*SD*= 0.52), 2.04 (*SD*= 0.50) for the sweet, sour, salty, bitter, and saccharin flavoured mixtures respectively. On stress days the mean avidity scores were 1.17 (*SD*=1.10), 1.80 (*SD*= 0.60), 1.36 (*SD*= 0.59), 1.07 (*SD*= 0.41), 1.40 (*SD*= 0.49) for the sweet, sour, salty, bitter, and saccharin flavoured mixtures respectively.

The avidity scores for the LoS rats were as follows. On control days the mean avidity scores were 2.07 (*SD*= 0.32), 2.20 (*SD*= 0.54), 2.41 (*SD*= 0.40), 2.17 (*SD*= 0.52), 2.50 (*SD*=0.50) for the sweet, sour, salty, bitter, and saccharin flavoured mixtures respectively. On stress days the mean avidity scores were 2.76 (*SD*=1.10), 1.19 (*SD*= 0.60), 1.29 (*SD*= 0.59), 1.06 (*SD*= 0.41), 1.16 (*SD*=0.49) for the sweet, sour, salty, bitter, and saccharin flavoured mixtures respectively.

When using preference scores, the hypothesis was still not supported. The three way interaction of taste by stress by phenotype was found to not be significant (F (4, 9) = 0.31, p> .05). The preference scores for the HiS rats were as follows. On control days the mean preference scores were 0.46 (*SD*=0.07), 0.40 (*SD*= 0.08), 0.44 (*SD*=0.10), 0.57(*SD*= 0.08), 0.42(*SD*= 0.08) for the sweet, sour, salty, bitter, and saccharin flavoured mixtures. On stress days the mean preference scores were 0.39(*SD*=0.07), 0.45 (*SD*= 0.08), 0.43 (*SD*= 0.07), 0.44 (*SD*= 0.06), 0.52 (*SD*=0.10) for the sweet, sour, salty, bitter, and saccharin flavoured mixtures.

The preference scores for the LoS rats were as follows. On control days the mean preference scores were 0.67 (*SD*= 0.07), 0.52 (*SD*= 0.08), 0.53 (*SD*=0.10), 0.52 (*SD*= 0.08), 0.50 (*SD*=0.08) for the sweet, sour, salty, bitter, and saccharin flavoured mixtures. On stress days the mean preference scores were 0.51(*SD*=0.07), 0.47 (*SD*=0.08), 0.62 (*SD*=0.07), 0.46 (*SD*=0.06), 0.50 (*SD*= 0.10) for the sweet, sour, salty, bitter, and saccharin flavoured mixtures.

4.8 Weight

Body weight (grams) of each subject was monitored daily during the data collection period of the experiment. Weight measurements were taken to ensure that the food deprivation required in the experiment was not resulting in any adverse health effects in the subjects. The weight measurements can be found in Appendix C.

4.9 Behavioural Observations

Behavioural observations were taken to determine if the subjects' behaviours varied from control to stress trials and to monitor the health status of the animals. The behaviours measured include rearing, licking, scratching, sniffing, being mobile/walking, grooming, being frozen/ doing nothing, biting anything, and eating behaviours. One-tailed paired samples t-tests were

performed to determine if the behaviours occur significantly more during stress as compared to control. However, the t-test for eating was performed to determine if eating was observed more during control as compared to during stress. Previous literature supports the notion that coping behaviours should increase during stress compared to during control; this results in decreased consumption during stress as compared to control which should be evidenced behaviourally with eating being observed less during stress compared to control (Krebs et al., 1996; Krebs et al., 1997). The results of these t-tests, and other descriptive statistics, can be seen in Table 4. Although none of the p-values were significant the p-values for eating (p=.06), sniffing (p= .05), and biting (p=.09) approach significance. However, it should be noted that biting only occurred 3 times and thus cannot be interpreted to represent the behaviour of the entire sample.

Table 4: Behaviours Exhibited During Control and Stress

	Nothing	Rearing	Walking	Eating	Grooming	Sniff	Dig	Lick	Bite	Scratch
Stress (%)	48.17	48.48	60.00	47.62	55.34	62.16	0	52.38	100	83.33
Ctrl (%)	51.83	51.51	40.00	52.38	44.66	37.84	100	47.62	0	16.67
Stress count	265	39	19	258	75	50	0	11	3	5
Ctrl count	275	37	14	285	71	30	2	10	0	1
Total count	438	66	25	462	103	74	2	21	3	6
P value *	0.30	0.38	0.12	0.06	0.38	0.05	0.08	0.40	0.09	0.13

**One-tailed significance value.*

5 Discussion

The HiS rats were found to have greater preference scores for a liquid saccharin solution during a two bottle preference test compared to the LoS rats; this difference approached significance (p=.06). It is likely that this result fell short of significance due to the small sample size of each strain (n = 7). However, the study continued under the assumption that the difference observed in preference scores between the HiS and LoS strains in the current study is real. This finding is in line with the previous literature regarding the HiS and LoS rat strains, thus the rats used in this study were accurately categorized as either a HiS rat or a LoS rat (Dess, 2000; Dess & Minor, 1996; Nachman, 1959). Additionally, both strains were found to significantly decrease their food consumption during stress; this behaviour was previously observed only in the normal rat population and the LoS strain, not the HiS strain (Dess and Minor, 1996; Krebs et al., 1996). Also, regardless of stress condition, the LoS rats were found to have significantly greater preference scores for the sweet solution as compared to the HiS rats. This result was unexpected and contrary to the existing literature (Dess, 2000). The observed difference in the preference for sweet between the HiS and LoS strains is the opposite of what was initially predicted, and none of the other predicted differences between the two strains for any of the other flavours were found. Additionally, predictions regarding the effects of stress on taste preferences and this effect being dependent upon the rat strain were not supported by the results. Although these findings did not support the sensation seeking model that was initially posited, these findings are still very informative. The unexpected results of the current study, namely that the HiS and LoS rats did not differ on taste preferences apart from sweet which was opposite the predicted direction, suggest that some variable unique or new to the current study (compared to the previous literature) has a significant influence on the consumptive behaviours of the HiS and LoS rats.

Specifically, this new variable (or new variables) influenced the consumptive behaviour of the HiS and LoS rats so strongly that the predictions made based upon the previous literature were not supported in the current study. This suggests that the many factors that influence consumptive behaviour may interact with one another, resulting in changes in behaviour. The different factors possibly at play such as the effects of satiety on taste preference, and the influence of calories and texture on consumption will be addressed as the predictions and corresponding results are further discussed.

5.1 Confirming characteristics of the phenotypes

The HiS and LoS strains are defined by their specific preference scores for a saccharin solution. Namely, the HiS rats have a greater preference for a saccharin solution compared to the LoS rats (Dess & Minor, 1996; Nachman, 1958). The results of the current study support this finding with a one-tailed independent samples t-test that approaches significance. A one- tailed independent t-test was used instead of a two-tailed t-test because literature and theory strongly supports a significant difference in saccharin preference between the two strains, thus a one-tailed t-test can be justified (Field, 2009). A one-tailed t-test has more statistical power than a two-tailed one (ability to correctly reject a false null hypothesis), which was useful as this helps to compensate for a small sample size (Field, 2009).

It also is noteworthy that the HiS and LoS strains of the current study obtained preference scores above 0.50, indicating that each strain preferred the saccharin solution but the HiS rats (*M*=0.81) had greater preference scores compared to the LoS rats (M= 0.71). This is unlike the HiS and LoS rats originally discovered by Nachman (1959). In the Nachman (1959) study the HiS rats (*M*= 0.82) had preference scores above 0.50, indicating a preference for the saccharin

solution. On the other hand the LoS rats (*M*=0.40) had preference scores below 0.50, indicating an aversion to the saccharin solution. This discrepancy brings into question whether two different strains of rat were present in the current study, or more specifically, if the LoS rats were present in the current study.

Although the preference scores of the strains in the current study do not correspond with what was found in the Nachman (1959) study, they do correspond to the preference scores of the strains as they exist today in other more current studies (Dess & Minor, 1996). For example, in the study by Dess & Minor (1996) the strains were confirmed using a comparison of preference scores; the HiS rats (*M*=0.98) were found to have greater preference scores than the LoS rats (*M*= 0.83). However, both strains were found to prefer the saccharin solution with preference scores above 0.50. These scores are comparable to the scores of the rats used in the current study. Therefore it is likely that the current study does have both HiS and LoS strains of rat.

The rats used in current literature, and the rats originally discovered by Nachman (1959) may differ from one another because the Nachman (1959) study used a different strain of rat (Wistar rats) compared to the colony of HiS and LoS rats that exist today (Sprague Dawley) (Carroll et al., 2008; Nachman, 1959). Additionally it is possible that the HiS and LoS rats obtained for the current experiment were not the best representatives of the strains (i.e. The HiS rats had a lesser preference for saccharin than expected and/or the LoS rats had a greater preference than expected) and did not differ as much as would have been expected. This is compounded by the fact that only a small sample size (*n*= 7) could be obtained of each strain, which could be why the strain difference fell short of obtaining significance.

The HiS and LoS rats used in this study were also found to significantly differ in body weight. As was previously stated in the results section, this is not an uncommon finding in the literature among the female rats (Dess & Minor, 1996). However, this difference in weight between the HiS and LoS rats may have influenced the difference in the preference scores of the HiS and LoS rats for the sweet test food. The LoS rats were found to have significantly greater preference scores for the sweet test food compared to the HiS rats, which is opposite of what the literature indicates (Dess, 2000). The implications of the weight difference between the HiS and LoS rats will be further elaborated upon later in the discussion.

5.2 Line differences in flavour consumption

The avidity scores of the differently flavoured test foods (sweet, sour, salty, bitter, saccharin) were not found to significantly differ between the HiS and LoS rats. This result differs from the hypothesized result; it was predicted that the HiS strain would consume significantly greater amounts of the sweet, salty, and saccharin mixtures as compared to the LoS rats, and that consumption of the sour and bitter mixtures would not differ between the two strains. Thus the consumption (avidity scores) of different tastes was not found to differ between the HiS and LoS strains.

On the other hand, when looking at preference scores significant results were found. The LoS rats were found to have greater preference scores for the sweet test food as compared to the HiS rats, and did not differ in consumption of any of the other flavours (Figure 2). These results partially support the hypothesis. The prediction that the HiS rats would have greater preference scores for the sweet, salty, and saccharin test foods compared to the LoS rats was not supported. The HiS and LoS rats did not significantly differ in consumption of the salty and saccharin

flavoured food, and although the two strains did significantly differ in consumption of the sweet food it was the LoS rats with greater preference scores for the sweet test food compared to the HiS rats (unlike what was predicted). However, the predictions that the HiS and LoS rats would not differ in their consumption of the sour and bitter test foods was supported by the results.

If the sensation seeking model was supported, the HiS rats would have shown greater preference and avidity scores for the sweet, salty, and saccharin flavoured mixtures compared to the LoS rats and there would have been no difference in preference and avidity scores for the sour and bitter mixtures between the two strains. Because, as sensation seekers, the HiS rats would want to consume more of the pleasantly flavoured mixtures for the stimulation it would provide. This is in contrast with the LoS rats who, as sensation non-seekers, would try to limit any extra stimulation. In the proposed model, sensation seekers and non-sensation seekers were expected to not differ in consumption of the aversively flavoured foods because survival instincts would equally encourage both strains to avoid the sour and bitter foods (Myers & Scalfani, 2006; Tordoff, Alarcon, & Lawler, 2008; Pinel, 2009).

However, the results did not align with the sensation seeking paradigm and its predictions. This suggests that the sensation seeking model is not the best way to frame the results of this study. This could be because the sensation seeking model does not take into account some of the unique attributes of the current study when compared to the existing literature. For example, the current study uses flavoured solid food, compared to the previous literature with the HiS and LoS strains that used flavoured liquids to measure the taste preferences of these rats (Dess, 2000). In the study by Dess (2000), two bottle preference tests were performed with the HiS and LoS rats using various flavoured liquids. It was found that the HiS line had greater preference scores for the hedonic liquids (sweet, and salty) compared to the

LoS line. The strains did not differ in preference for the aversive liquids (sour and bitter). However, the behaviour of the animals was different in the current study when using flavoured solids to test the rats' taste preferences.

The results in the current study most likely resulted from the use of flavoured rat food (taste and calories delivered as a solid) compared to the Dess (2000) study which used flavoured liquids (taste and no calories delivered as a liquid), as this was one of the most prominent changes between the two studies. Many bodily changes and signals occur during the consumption of food that can affect both satiety and preference for different flavours (Frank et al., 2013; Scalfani, 2013; Woods, 2013). Some of these bodily changes and sensations/signals include changes in the levels of satiety/hunger hormones, the perception of calories, taste and texture of the food being eaten. All of this information integrates within the PGC (Frank et al., 2013; Goldstein, 2010; Scalfani, 2013; Woods, 2013). The integration of this information in the PGC may be the cause of the changes to consumption and food/ taste preferences that have been seen in the previous literature (Berridge, 1991; Griffioen et al., 2012; Holman, 1969; Laeng et al., 1993; Moskowitz et al., 1976; Rolls et al., 1988). Specifically, with regard to the current study, because it is known that all of these signals are integrated, and that each of these integrated signals on its own influences consumptive behaviour, it is reasonable to posit that the change in texture and caloric content of the test foods as well as the addition of a restraint stressor in the current study in comparison to the Dess (2000) study may have affected the integrated signal in the PGC thus resulting in changes in consumptive behaviour in the current study compared to the previous literature.

A wealth of literature exists detailing the effects of stress, caloric content and the texture of food on consumptive behaviour (de Wijk et al., 2008; Geliebter, 1979; Hogenkamp et al.,

2012; Krebs et al., 1996; Macht et al., 2001; Naim et al., 1986; Young,et al., 1974; Zijlstra et al., 2008). For example, rats have been found to prefer small pellets of rat food compared to large pellets or powdered options of the same food (Naim et al., 1986). It has also been shown that regardless of caloric and nutritional content, a liquid product is eaten in larger amounts compared to a semi- solid product. It has been suggested that liquids are less satiating than solids, and as a result liquids are consumed in greater amounts despite caloric or nutritional content (de Wijk et al., 2008; Hogenkamp et al., 2012; Zijlstra et al., 2008). The literature has also shown that the caloric content of food influences consumptive behaviour. The presence of calories in food has been found to be necessary to achieve satiety, and hyperphagia results when calories are removed (Geliebter, 1979; Young et al., 1974).

Thus the literature shows that calories and food texture have a significant influence on the consumptive behaviour of rats (de Wijk et al., 2008; Geliebter, 1979; Hogenkamp et al., 2012; Naim et al., 1986; Young,et al., 1974; Zijlstra et al., 2008). With regard to the LoS rats having a greater preference for the sweet test food compared to the HiS rats in the present study but the opposite being found in the Dess (2000) study for a sweet flavoured non-caloric liquid, the presence of calories and the use of a test food (instead of a test liquid) most likely caused this discrepancy in results between the two studies. However, it is more ecologically valid to use foods. The biggest methodological difference between the current study and the Dess (2000) study is the use of a flavoured food instead of flavoured liquids; thus it is reasonable to postulate that it was the addition of calories and the change in texture that resulted in the unexpected results.

For example, calories may have had a particularly large role in the result of this interaction because calories are so necessary to survival and therefore are reinforcing. Therefore

it is possible that the presence of calories in the test foods was more reinforcing than the specific flavours of the foods. If the reinforcing quality of calories overshadowed the reinforcing qualities of the different flavours of the test foods, this may be why the expected effects of taste on consumption and the expected strain differences in taste preferences were not found.

Another possible explanation for why the LoS rats showed greater preference scores for the sweet food compared to the HiS rats is the fact that the LoS rats used in this study were significantly heavier than the HiS rats. The LoS rats have been found to be heavier than the HiS rats in previous literature and this is commonly seen amongst female rats (Dess & Minor, 1996). With the LoS rats being heavier, it makes sense that they would consume more calories than the HiS rats. In an effort to consume as many calories as possible, the LoS rats may be more motivated to consume greater amounts of the sweet food because sweetness tends to signal that a food is high in calories (Myers & Scalfani, 2006; Pinel, 2009). This would also explain why only the analysis using preference scores was significant and not the avidity scores, because avidity scores control for differences in weight.

It is also possible that the addition of calories and the weight difference between the two strains interacted to result in the LoS rats consuming a larger amount of the sweet food compared to the HiS rats. Specifically, the LoS rats are heavier and therefore have a greater appetite and consume more calories than the HiS rats. And the sweet food not only contains calories but is sweet flavoured (a flavour which signals the presence of calories). Therefore, it is possible that the LoS rats were more motivated to consume the sweet food because they have larger appetites compared to the HiS rats and because the sweet food contains more calories (or is flavoured in a way that suggests it contains more calories).

5.3 Effects of stress on overall consumption

The main effect of stress on consumption was found to be significant when using avidity scores (Figure 3), and was not found to be significant when using preference scores. Avidity scores were found to be significantly lower during stress as compared to during control. This means that when differences in weight among the rats are controlled for, the animals are found to eat significantly less during stress as compared to the control condition. This is in line with the hypothesis which predicted that stress would result in an overall decrease in consumption as compared to consumption in the absence of stress. This finding has been reported in the previous literature with studies using the normal rat population (Howell et al., 1999; Krebs et al., 1996). It has been theorized in the literature that consumption decreases during times of stress because coping behaviours take precedence over eating. During stress, the body's main concern is using available resources to re-establish homeostasis and/or keep the organism safe (Krebs et al., 1996; Krebs et al., 1997; Lovallo, 2005). The behavioural measures taken in the current study modestly support this notion. Sniffing behaviours were found to occur more during stress compared to control with a p-value approaching significance (p=.05). Eating behaviours were also observed more during control compared to stress, however not significantly (p=0.13). These two values support the notion that coping behaviours take precedence over eating behaviours during times of stress resulting in the decreased consumption during stress that has been reported in the literature (Krebs et al., 1996; Krebs et al., 1997; Lovallo, 2005).

These results provide evidence that the acute stressor used in the current study was effective at inducing a stress response in the rats thus resulting in the behavioural changes observed in the rats; namely a decreased consumption and likely increase in the occurrence of coping behaviours during stress as compared to in the absence of stress. This finding is in line

with the previous literature by Krebs et al. (1996; 1997); where consumption of the normal rat population decreased during stress when a restraint stressor was used and that an increased coping behaviours during stress was the cause.

As previously stated, the sensation seeking model predicts that while consumption would decrease overall in response to stress, the effects of stress would be exaggerated in the LoS population as compared to the HiS population. For example, if stress caused a decreased consumption, this effect would be greater for the LoS rats as compared to the HiS rats. In accordance with the sensation seeking model, the non-sensation seekers are said to be more reactive and sensitive to stress. Thus it was expected that however stress affected the sensation seekers (HiS rats), the non-sensation seekers (LoS rats) would be affected in the same manner, but to a larger degree. However, this prediction was not supported by any of the results; the LoS rats did not decrease their consumption during stress to a larger degree than the HiS rats. In other words, the stress by phenotype interaction (when using both avidity and preference scores) was not found to be significant. This means that the effect of stress on the consumption of the animals did not differ between the HiS and LoS rats. Thus the LoS rats were not found to be more reactive to stress when compared to the HiS rats.

Although the current study did not find the LoS rats to be more sensitive to stress compared to the HiS rats, the previous literature does suggest that stress affects consumption of the HiS and LoS rats differently. As reviewed earlier, Dess and Minor (1996) found that when the HiS and LoS rats were stressed the LoS rats significantly reduced their food intake compared to consumption in the absence of stress. On the other hand, the HiS rats did not exhibit any changes in their consumption patterns during stress. This difference in reactions during stress could be rooted in different arousal levels of the HiS and LoS rats; specifically that the LoS rats

have a higher baseline corticosterone (a stress hormone) level than HiS rats (VanderWeele, Dess, & Castonguay, 2002). Although the Dess and Minor (1996) study provided evidence for a differential effect of stress on consumption of the HiS and LoS strains, some methodological differences between the Dess and Minor (1996) study and the current study may have led to the different results.

For example, the Dess and Minor (1996) and the VanderWeele, Dess, and Castonguay (202) studies each used a 0.6 mA tail shock as the stressor, whereas the current study employed an acute restraint stressor. The restraint stressor was chosen because the previous literature indicates that both acute restraint stress and tail shock decrease consumption during stress. Thus it was reasoned that the effects of each would be comparable (Krebs et al., 1996). Additionally, using a different stressor in this study was a methodological strength as it allows for the observation of the effects of a different stressor on the consumption of the HiS and LoS rats. This endeavor proved to be informative considering that the results of the current study suggest that the two different stressors do not affect the rats the same way. It would appear that the tail shock stressor affects the HiS and LoS rats in a manner that results in different behaviours from the two strains. On the other hand, the acute restraint stressor did not differentially affect consumption between the two strains, even though it did significantly decrease the rats' consumption.

It is possible that the distinguishing variable between the two stressors is that a tail shock involves pain. Perhaps the HiS and LoS strains differ in their sensitivity to pain, and it was this difference in the Dess and Minor (1996) study that resulted in the differential effect of stress on the consumption of the two strains. In contrast, the current study used a restraint stressor that inflicts no pain on the animal, resulting in no difference in consumption between the HiS and LoS strains during stress. In fact this theory is in line with the sensation seeking paradigm. Non-

sensation seekers have been found to be more sensitive to pain, thus this difference in the perception of pain by sensation seekers (HiS rats) vs. non sensation seekers (LoS rats) could have resulted in the differential reaction of the HiS and LoS strains to the tail shock stressor in the Dess and Minor (1996) study (De Pascalis et al., 2007). Thus the current study provides evidence that suggests the HiS and LoS strains do not differ in their consumption response to stress, provided that the stressor used does not inflict pain on the subjects.

5.4 Differential consumption of flavours during stress

Another finding of the present study was that stress did not influence the consumption (both preference and avidity scores) of any of the different flavours. Specifically, consumption of the various five flavours (sweet, sour, salty, bitter, saccharin) did not differ during the stress condition as compared to the control condition. These results partially supported the hypothesis. It was predicted that the consumption of the different flavours would vary depending upon the stress condition. Specifically, it was predicted that the consumption of hedonic tastes (sweet, salty, and saccharin) would increase during stress as compared to control and the consumption of anhedonic tastes (sour and bitter) would remain the same or decrease during the stress condition as compared to the control. The avidity and preference score results led to the same conclusions. The prediction regarding anhedonic tastes was supported with the consumption of the sour and bitter tastes remaining the same between the control and stress condition. On the other hand the prediction regarding the hedonic tastes was not supported; the consumption of hedonic tastes did not increase during stress as compared to the control condition. However the statistical analyses were not significant. Therefore, although the preference for bitter and sour tastes did not differ significantly between stress and control, the interaction itself was not significant and one cannot

draw any meaningful conclusions from a null hypothesis. Therefore the hypothesis for the stress by taste interaction was not supported.

The existing literature details many studies where stress successfully influences the taste preferences of the normal rat population (Dess, 1991; Christiansen et al., 2011; Howell et al., 1999; Tarjan & Denton, 1990). Consumption of sucrose (sweet) has been found to increase during times of stress (Christiansen et al., 2011); evidence suggests this occurs because the consumption of sucrose attenuates the HPA axis response during stress, and thus is a rewarding behaviour (Christiansen et al., 2011). The same effect has been documented with the consumption of sodium chloride during stress (salty) (Howell et al., 1999; Tarjan & Denton, 1990). In contrast, the consumption of aversive flavours (sour and bitter) in the literature has been found to either remain the same, or to decrease even further (Howell et al., 1999). It has been theorized that this is because it is beneficial to survival to avoid sour and bitter tastes regardless of stress as these flavours often indicate foods that are toxic or spoiled (Naim et al., 1986). Additionally, stress may make the animals more sensitive to these tastes, and thus at times decreasing consumption even further (Dess, 1992; Howell et al., 1999). A similar phenomenon has been found to occur with saccharin which is a unique taste as it has both hedonic and aversive qualities (a bitter-sweet taste). Despite its hedonic qualities, consumption of saccharin has been found to decrease during stress, possibly due to an increased sensitivity during stress to the bitter quality of the compound (Dess, 1992).

Many of the above mentioned findings, such as salty foods being consumed more during stress or saccharin being consumed less during stress, were not replicated in the current study. This may be due to a few different reasons. Firstly, each of the previously reviewed studies used flavoured liquids, whereas the current study used flavoured rat chow. As was already discussed,

there is existing literature showing that satiety and consumption of foods with different textures, or caloric contents may influence taste/ food preferences as well as consumption (Berridge, 1991; de Wijk et al., 2008; Geliebter, 1979; Griffioen et al., 2012; Hogenkamp et al., 2012; Holman, 1969; Laeng et al., 1993; Moskowitz et al., 1976; Naim et al., 1986; Rolls et al., 1988; Young et al., 1974; Zijlstra et al., 2008). The calories/ food consumed affect consumption and taste preference by changing gut hormone levels. This change in gut hormones is detected and relayed to the PGC by the vagus nerve resulting in a change in the activation of the PGC, an organ which integrates caloric, texture, and taste information of the food being eaten as well as emotional and cognitive information (Frank et al., 2001; Goldstein, 2010; Scalfani, 2013). It is possible that the integration of the emotional state of the animals (the effect of the stressor), in addition to the integration of the information regarding the texture of the calories contained in the test foods in the PGC may have influenced the consumptive behaviour of the HiS and LoS rats (Frank et al., 1002; Goldstein, 2010; Scalfani, 2013).

For example, in the previous studies without calories the preferences of the animals was based purely upon the flavour of the test solution (Dess, 2000). The flavours salty and sweet signal the presence of nutrients and calories and were therefore preferred. On the other hand flavours that signal that a food may be poisonous or spoiled, such as bitter or sour, were not preferred (Myers & Scalfani, 2006; Pinel, 2009). Because the consumption of salty and sweet foods is often accompanied by nutrients and calories, the consumption of these types of foods stuffs is rewarding and thus the behaviour (consuming substances that are sweet or salty) is reinforced. However, calories were present in the test solutions of the present study and calories are also reinforcing. Therefore it is possible that the presence of calories in the test foods was

more reinforcing than the flavours of the foods. This could result in the removal of any of the taste preference differences that were seen in the Dess (2000) study.

Another possible reason for the unexpected results is the fact that the studies mentioned earlier regarding taste preferences during stress all used the normal rat population as their subjects (Dess, 1991; Dess & Minor, 1996; Christiansen et al., 2011; Howell et al., 1999; Tarjan & Denton, 1990). Considering that the defining feature of the HiS and LoS strains is that they were bred to have specific taste preferences for saccharin, it is logical to assume that differences in preference for other tastes and reactivity to stress may have been selected for. Thus the fact that many of the predictions were made in consideration of literature that used the normal rat population and not the HiS and LoS strains may be the reason that the results do not fall in line with the literature.

Although the taste by stress interaction was not found to be significant, it should be noted that the avidity score results regarding this interaction displayed a lot of variance between stress and control groups per flavour (Figure 4), despite being a non-significant result. In an effort to be thorough, paired samples t –tests were performed to examine these differences. With the exception of the sweet mixture, all of the flavoured mixtures displayed smaller avidity scores during stress compared to control. This is to be expected considering that consumption during stress was found to significantly decrease compared to control conditions. The interaction may not have been significant because the variance was too great, thus it would be beneficial to perform this study with a larger sample size to help decrease the standard error measure.

However, it is noteworthy that of all the flavoured test foods only the sweet food (sucrose) did not display decreased consumption during stress as compared to control. This

suggests that something about the addition of sucrose to the rat chow has resulted in a minimized (possibly eliminated) effect of stress on consumption. Previous literature displays evidence that sucrose is preferred during stress because the consumption of sucrose has been found to attenuate the HPA axis response by decreasing plasma corticosterone levels; thus it is reinforcing to consume sucrose for its calming effect on the HPA axis response (Christiansen et al., 2011).

If the sucrose contained in the sweet test food reduced the stress hormone levels of the animals, then it is possible that the various behaviours associated with stress (decreased consumption, dilated pupils, accelerated heart rate, etc.) would decrease as well. Therefore, if the stressor decreased consumption (as evidenced with the other flavours) while the sucrose in the test food simultaneously increased consumption (because consumption of sucrose is rewarding, especially during stress), it is possible that the effect of both the stressor and sucrose consumption cancelled each other out. This would result in no significant difference in the consumption of the sweet test food during stress compared to during the control condition.

5.5 Effects of stress on flavour consumption of the LoS rats compared to the HiS rats

And finally, the last result in the current study was that the taste by strain by stress interaction was not significant. This means that consumption of the differently flavoured test foods was not different during stress compared to during control and that the effect of stress on the consumption of the differently flavoured test foods was no different for HiS rats compared to LoS rats. This result differs from the prediction which was that the effects of stress on the consumption of the different flavours would be exaggerated in the LoS population compared to the HiS population. For example, if stress were to result in an increased consumption of hedonic tastes and a decreased consumption of anhedonic tastes, the HiS and LoS rats would both exhibit

these changes but this effect would be greater for the LoS rats. However the taste by stress by strain interaction was not significant and thus the results do not support this prediction. If the results were as predicted by the sensation seeking paradigm, the sensation seekers (HiS rats) should have shown an increased preference for the hedonic tastes (sweet, salty, saccharin) during stress as compared to control and either no change or a decreased consumption of anhedonic tastes (sour, bitter) during stress compared to control. The non-sensation seekers (LoS rats) would have also shown this behaviour but to an even greater degree. This is because the non-sensation seekers are more sensitive to the stressor than the sensation seekers and thus the effects of the stressor on their behaviour would be exaggerated. However, the data does not fit this prediction which suggests that the sensation seeking paradigm is not a sufficient model to explain the results of the current study. The results of the current study suggest that factors other than optimal arousal are involved which is influencing the results in ways that were not predicted.

As was stated earlier, previous literature regarding the changes in consumption during stress of the HiS and LoS strains is limited. What has been found suggests that the LoS rats are indeed more sensitive to stress and that the consumption of a saccharin solution by both the HiS and LoS strains is equally sensitive to the effects of stress (Dess & Minor, 1996). The experiment detailing how stress differentially affects the amount consumed by the HiS and LoS strains was described earlier. It is likely that the LoS rats decreased their food consumption during stress and the HiS rats did not in the previous literature because the Dess and Minor (1996) study used a tail shock. Thus that experiment was measuring a difference in response to pain, not stress. The results of the current study provides evidence that the HiS and LoS rats both decrease their

consumption during stress and thus do not differ in their eating responses to stress, provided that the stressor being used does not cause pain.

With regard to how stress influences taste/food preferences, the Dess and Minor (1996) study also detailed an experiment that looked at the effect of stress on the preference of the HiS and LoS strains for saccharin. The current study predicted that saccharin was a hedonic taste and thus consumption of it would increase during stress, and that this effect would be exaggerated in the LoS population due to their increased sensitivity to stress. Again, the difference in stressors used between this study and the previous literature may be the cause of the discrepancy in the results.

Again the methodologies of the previous literature use flavoured liquids instead of flavoured foods when determining how stress influences taste preferences. Thus, the addition of calories to the test solutions could have influenced the taste preferences in difficult to predict ways, resulting in the unexpected results of this study (a more detailed explanation can be seen earlier in the discussion). Thus the results of this study were informative, with the results supporting the notion that calories and texture influence consumption to such a large degree that the addition of these variables to the test foods in the current study caused the results to differ from the Dess (2000) study that used flavoured water (a zero calorie liquid).

5.6 Limitations and Future Directions

As with any study there are always areas which can be improved upon or which limit how the results may be generalized. For example, it is a limitation that the strain difference in preference score approached, but did not attain significance. Therefore, it is possible that the reason the hypotheses of the current study were not supported is because the two groups did not

differ from one another. However, the preference scores of the strains in the current study are similar to preference scores found in previous literature with these rats (Dess & Minor, 1996). This supports the notion that two different strains of rats were indeed present. If that is the case, then it is likely that significance for the strain difference fell short due to the small sample sizes (n=7) used. Smaller sample sizes were used because these animals are difficult to obtain (specialized breed), and the initial sample size was reduced due to health issues.

Therefore in the future it would be informative to use a larger sample size. As with most studies, a larger sample size could be beneficial as it would help to decrease the measures of error variance which would improve the power of the hypothesis tests (Field, 2009). Additionally, a visual inspection of the graphs revealed some significant simple effects even though the interaction was not significant (Figure 4). These issues may stem from a large error variance, which can be minimized by increasing the sample size. It is also noteworthy that many of the significance values of the current study were near significant, an increase in sample size (and thus a decrease in the error variance) could help to push these near significant values past the threshold for significance.

Another limitation is that in the conclusions made regarding the activation of the PGC must be made with caution as the current study does not specifically measure brain activation or changes in hormone levels of the subjects. Additionally, the current study only uses one type of stressor (a restraint stressor), therefore generalizing the effects of stress seen in the results of the current study to the human population or making conclusions regarding the effects of different types of stressors on the HiS and LoS populations must be done carefully. This is because a restraint stressor may differ from the various types of stressors humans experience in their day to day lives and different stressors may affect the HiS and LoS strains in different ways compared

to a restraint stressor. The literature supports the notion that not all stressors and its effects are equal (Krebs et al., 1996).

Another possible area for improvement in the current study would be the use of both male and female rats in the future so that any gender differences in the influence of stress on consumption in the HiS and LoS strains may be investigated. Female rats were chosen to keep the variable of gender stable between the HiS and LoS groups of rats, however it may be fruitful to investigate if gender influences how stress effects the taste preferences of the HiS and LoS strains.

With regard to future directions, performing the current study with different conditions could be informative as it would help to garner a better understanding of the different variables at play. For example, the influence of texture on the results of the present study could be investigated by replicating the current methodology but with solid food instead of the moistened crushed rat chow used in the present study. Moistened crushed rat chow was used in the current study because adding a flavoured liquid to the powdered rat chow was a flavouring method that ensured uniformity in taste of the product. If the powdered rat chow is moistened with flavoured liquid and all of the chow is wet, then it can be assumed with high confidence that whatever tastant is being added to the food can be detected equally throughout the entire product.

However, it should be noted that the literature has shown that rats prefer to eat food that is solid and of the size that can be handled easily (such as the size of conventional rat chow pellets) (Naim et al., 1986). Although the texture of the food was not a confounding variable, it may have reduced the consumption of the all the animals thus reducing the effect size of the results. Thus it would be beneficial for the methodology of this study to be replicated with solid

(not crushed and moistened) flavoured food. For example, Research Diets Inc (2014) provides customizable rat chow pellets where various qualities of the rat food can be controlled (calorie content, nutrient content, additional compounds). With this service a rat pellet could possibly be designed to fulfill all the necessary dietary needs, but with the addition of specific compounds in order to change the taste. This would present the test foods to the subjects in their preferred format (medium sized solid pellets).

The current study differs from all the previous literature regarding taste preferences of the HiS and LoS strains because the current study used flavoured food, instead of flavoured liquids as in the Dess (2000) study. Flavoured foods were used in an attempt to have a more ecologically valid analogue of consumptive behaviour. With the introduction of the new variables (calories, texture, stress) into the methodology, the effects seen in the previous literature have been taken away, such as the HiS and LoS rats having greater preference and avidity scores for the flavours of sweet, salty, and saccharin (Dess, 2000). This suggests these new variables, which have been found to individually influence consumptive behaviour (and which are found to integrate their signals in the PGC (Berridge, 1991, Griffioen et al., 2012; Holman, 1969; Laeng et al., 1993; Moskowitz et al., 1976; Rolls et al., 1988), interact with one another to influence consumptive behaviour together.

This is an important finding; therefore it would be beneficial in the future to investigate further. This could be accomplished by repeating the methodology of the current study with flavoured caloric liquids instead of flavoured foods. This would allow for an investigation of how stress influences taste preferences, without the possibility of issues regarding hunger, food texture, and satiety influencing the results. Thus a study which more closely resembles the Dess (2000) methodology, but with the addition of a stressor and calories could be a fruitful endeavor

as it would help to parse out the effect of stress on the consumptive behaviour of the HiS and LoS strains.

Performing the above suggested study could help in identifying what role the presence of calories played in the findings of the current study by a comparison the findings from the current study with the findings of the study being suggested for the future. It could also be informative on the influence of texture on the results of the current study. As mentioned previously, solid foods cannot be consumed in such large amounts as liquids can be, therefore a ceiling effect may be reached. The use of flavoured caloric liquids helps to remove this ceiling effect so that the results may be more accurately compared to the results of previous research on the taste preferences of the HiS and LoS strains.

5.7 Summary and Conclusion

To summarize, the current study was undertaken with the intention of identifying the food/ taste preferences of the HiS and LoS strains and to then determine if stress affects these food/taste preferences. The predictions of this study were made from the perspective of the sensation seeking/optimal arousal paradigm. It was predicted that the HiS rats (sensation seekers) would behave in a way that gives them access to stimulating experiences and stimuli, and the LoS rats (non-sensation seekers) would behave in a way that avoids extra stimulation. With regard to consumption, this would manifest with the HiS rats consuming greater amounts of the hedonic test solutions and equal amounts of the anhedonic test solutions compared to the LoS rats. Additionally, the LoS rats were predicted to be more sensitive to stress; Thus it was predicted that whatever effect stress had on consumption, that effect would be exaggerated in the LoS rats compared to the HiS rats. For example, it was predicted that the strains would increase

their consumption of the hedonic tastes, and that consumption of the anhedonic tastes would decrease or remain the same during stress; therefore, this effect was predicted to be more pronounced in the LoS population.

Although the restraint stressor was successful in reducing consumption of both of the strains, the predictions regarding the sensation seeking paradigm were not supported. Mostly non-significant results were found, and the significant result that was found was not as predicted. The LoS rats were found to consume greater amounts of the sweet test solution compared to the HiS rats which suggests that some unaccounted for factors may be at play. Specifically, it was suggested that the addition of a flavoured food (calories/texture) in the current study instead of using a flavoured liquid as in the previous literature as well as the addition of a stressor, may have resulted in the changes in the results. Each of these variables has been found in the literature to individually influence consumptive behaviour (de Wijk et al., 2008; Geliebter, 1979; Hogenkamp et al., 2012; Krebs et al., 1996; Macht et al., 2001; Naim et al., 1986; Young,et al., 1974; Zijlstra et al., 2008). The literature also indicates that changes in each of these variables is processed and integrated within the PGC (Berridge, 1991, Griffioen et al., 2012; Holman, 1969; Laeng et al., 1993; Moskowitz et al., 1976; Rolls et al., 1988). Thus it is reasonable to posit that the addition of calories and a stressor, and the change in texture of the test foods all interacted to influence the consumptive behaviour of the HiS and LoS rats of the current study.

From the results of the current study, it can be concluded that although the restraint stressor was indeed effective and caused an overall decrease in consumption, there was no significant interaction between stress and flavour (the effect of stress on consumption was not dependent upon which flavoured food was being consumed). Additionally, the effect of stress on consumption did not differ between the two strains. It can also be concluded that the sensation

seeking paradigm does not adequately explain the behaviour of the HiS and LoS strains. It is more likely that the presence of calories in the test solutions caused physiological changes in the subjects which caused the results seen in the current study. The physiological changes which occur during consumption of the HiS and LoS strains could be further explored in future studies, and may provide insights regarding the physiological changes of humans during consumption.

6 References

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Appendix A: Stress and Control Day Counterbalancing Order

Control and Stress day Presentation Order

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
ID																				
01	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C
02	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
03	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C
04	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
05	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C
06	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
07	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C
08	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
09	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C
10	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
11	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C
12	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
13	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C
14	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S

Note: S=Stress day and C= Control day. ID= Animal identification number. Bolded ID numbers are HiS rats.

Appendix B: Flavour Presentation Order

Flavour Presentation Order

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
ID																				
01	Qu	Su	Ci	Sa	So	Qu	Su	Ci	Sa	So	Qu	Su	Ci	Sa	So	Qu	Su	Ci	Sa	So
02	So	Sa	Su	Ci	Qu	So	Sa	Su	Ci	Qu	So	Sa	Su	Ci	Qu	So	Sa	Su	Ci	Qu
03	Su	Ci	Qu	So	Sa	Su	Ci	Qu	So	Sa	Su	Ci	Qu	So	Sa	Su	Ci	Qu	So	Sa
04	Ci	So	Sa	Qu	Su	Ci	So	Sa	Qu	Su	Ci	So	Sa	Qu	Su	Ci	So	Sa	Qu	Su
05	Sa	Qu	So	Su	Ci	Sa	Qu	So	Su	Ci	Sa	Qu	So	Su	Ci	Sa	Qu	So	Su	Ci
06	Qu	Ci	Su	So	Sa	Qu	Ci	Su	So	Sa	Qu	Ci	Su	So	Sa	Qu	Ci	Su	So	Sa
07	So	Sa	Ci	Qu	Su	So	Sa	Ci	Qu	Su	So	Sa	Ci	Qu	Su	So	Sa	Ci	Qu	Su
08	Ci	Su	Qu	So	Sa	Ci	Su	Qu	So	Sa	Ci	Su	Qu	So	Sa	Ci	Su	Qu	So	Sa
09	Su	Qu	Sa	Ci	So	Su	Qu	Sa	Ci	So	Su	Qu	Sa	Ci	So	Su	Qu	Sa	Ci	So
10	Sa	Ci	Su	Qu	So	Sa	Ci	Su	Qu	So	Sa	Ci	Su	Qu	So	Sa	Ci	Su	Qu	So
11	Su	Sa	So	Qu	Ci	Su	Sa	So	Qu	Ci	Su	Sa	So	Qu	Ci	Su	Sa	So	Qu	Ci
12	Qu	So	Ci	Sa	Su	Qu	So	Ci	Sa	Su	Qu	So	Ci	Sa	Su	Qu	So	Ci	Sa	Su
13	So	Qu	Sa	Ci	Su	So	Qu	Sa	Ci	Su	So	Qu	Sa	Ci	Su	So	Qu	Sa	Ci	Su
14	So	Su	Ci	Sa	Qu	So	Su	Ci	Sa	Qu	So	Su	Ci	Sa	Qu	So	Su	Ci	Sa	Qu

Note: Su=Sweet food (Sucrose), So= Salty Food (Sodium Chloride), Sa= Saccharin Flavoured Food, Cit= Sour Food (Citric Acid), Qu= Bitter Food (Quinine).ID= Animal identification number. Bolded ID numbers are HiS rats.

Appendix C: Daily Body Weight Measurements

Daily Body Weight for Duration of Experiment

ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Day														
01	320.5	355.6	426.8	339.1	308.8	305.9	303.7	360.5	415.7	466.1	457.4	397.2	330.9	365.4
02	324.6	363.2	425.3	347.2	310.5	311.1	311.2	343.7	416.6	455.6	458.1	394.2	343.8	344.1
03	326.0	364.8	418.3	345.7	312.4	299.5	306.1	356.4	403.3	458.1	449.7	400.5	337.2	350.5
04	313.3	364.2	423.2	348.9	311.0	308.9	307.3	355.6	405.5	455.0	449.2	390.0	335.2	348.4
05	323.3	346.1	422.2	330.9	299.9	313.1	306.2	351.1	403.5	463.4	439.2	401.4	344.9	350.6
06	315.0	356.2	416.1	335.9	311.1	310.0	305.0	357.1	398.9	464.4	440.2	400.7	334.5	345.3
07	329.8	352.1	407.1	344.1	314.3	305.7	305.6	351.6	382.7	458.4	433.6	378.2	339.7	347.2
08	319.8	338.1	414.5	343.2	310.6	306.7	305.9	348.9	395.1	465.5	439.7	372.8	342.3	349.8
09	308.5	354.7	413.4	343.2	314.1	306.7	311.2	351.2	396.1	457.1	443.1	387.0	328.7	346.4
10	307.2	352.7	407.3	341.4	301.9	300.6	308.1	349.9	381.4	438.8	445.8	385.2	343.5	342.7
11	316.6	347.4	412.9	330.9	318.5	302.9	305.6	350.0	401.5	447.0	445.1	369.5	326.7	349.4
12	315.6	351.1	411.9	336.1	314.1	293.3	306.3	347.4	401.5	454.9	441.1	368.5	323.0	346.2
13	316.0	340.0	409.1	325.5	317.2	311.9	305.7	346.1	397.5	454.9	440.5	371.9	331.1	345.9
14	310.9	346.3	409.0	339.5	317.4	314.6	306.1	342.0	396.7	454.7	442.8	366.1	333.5	346.8
15	313.3	346.8	403.5	337.6	313.0	312.3	306.5	346.9	398.4	452.6	449.0	365.4	332.8	342.5
16	313.5	338.5	404.7	334.6	315.9	320.0	305.1	347.5	399.1	437.7	443.3	362.8	325.4	353.5
17	314.5	335.5	406.8	333.6	318.1	320.0	314.3	349.6	401.4	436.5	444.9	365.1	332.5	355.4
18	312.3	351.1	403.4	338.9	320.4	350.2	300.2	345.9	403.3	450. 5	427.4	367.4	330.4	355.0
19	310.0	347.2	406.4	339.8	312.5	317.1	314.5	358.0	398.5	452.0	418.4	363.4	337.7	354.1
20	312.6	343.8	404.5	334.8	310.1	314.7	318.5	357.7	393.9	452.5	419.3	369.6	342.0	350.0
21	317.1	360.0	404.0	336.6	312.9	325.1	335.0	364.8	412.3	461.3	431.7	384.8	340.0	356.7
22	312.5	356.9	404.6	331.7	312.9	324.0	333.7	360.0	411.5	464.5	434.1	384.7	341.5	354.4
23	318.2	357.4	407.6	333.5	316.0	327.6	335.0	365.0	404.9	470.0	435.7	387.4	350.0	357.6
24	315.6	356.9	406.6	331.9	316.7	326.8	337.0	363.2	402.3	464.4	435.5	385.9	348.0	357.3
25	317.3	354.9	410.5	336.5	316.4	327.8	341.1	367.3	395.5	464.6	436.2	386.0	353.7	353.2
26	319.2	357.3	408.4	331.5	313.5	334.7	342.0	370.0	397.5	463.7	442.2	391.8	350.0	353.5
27	315.6	350.9	404.8	330.0	313.9	335.6	335.5	353.4	392.3	458.5	431.1	390.0	342.5	352.5
28	315.9	355.5	403.9	330.8	310.0	333.5	334.3	361.5	399.0	462.0	433.5	392.2	343.5	347.2
29	317.6	353.2	404.3	332.0	311.7	334.6	335.1	357.6	396.6	460.0	432.2	391.8	343.5	348.0
30	322.7	358.5	400.0	330.0	313.5	323.5	328.8	363.8	391.9	459.5	432.2	387.5	336.7	347.8
31	320.5	355.7	400.0	332.0	314.0	322.9	329.1	360.4	392.6	459.6	433.1	384.4	340.0	348.9
32	315.4	363.1	406.1	336.9	325.9	327.5	337.9	369.4	400.0	460.7	334.9	385.5	342.8	357.2
33	312.2	343.1	397.6	322.6	325.5	318.5	331.7	355.5	382.6	443.0	422.5	390.0	342.6	352.4
34	310.0	346.1	394.5	332.0	329.1	324.5	334.4	365.5	380.0	357.3	414.2	385.0	345.5	348.5

35	311.0	345.4	395.2	331.6	330.5	320.5	340.1	365.1	380.0	455.4	415.7	386.4	347.1	345.6
36	312.4	344.6	394.3	331.5	325.6	328.1	332.6	369.2	369.7	447.4	419.0	381.6	330.0	346.9
37	311.4	343.8	392.3	324.0	327.4	318.6	331.5	365.1	376.9	435.5	420.0	378.3	329.1	347.2
38	311.6	344.6	393.0	324.3	328.4	320.4	331.5	364.8	377.4	435.0	419.1	379.1	330.8	345.5
39	309.5	340.6	388.5	330.0	327.5	312.2	330.9	357.3	372.5	434.1	422.9	371.6	330.5	348.1
40	301.6	343.1	381.1	338.2	321.0	217.5	326.2	364.9	366.3	442.8	410.0	380.0	328.8	345.8
41	305.6	339.8	380.0	327.2	327.2	322.0	336.5	364.9	370.0	437.4	416.9	376.6	327.0	345.5
42	305.0	343.5	376.3	337.2	323.9	330.0	336.8	365.8	364.9	436.7	403.9	383.4	328.4	349.0
43	309.5	346.4	376.9	328.3	334.0	317.6	343.5	367.3	368.6	430.0	403.6	384.6	336.3	346.5
44	310.0	348.5	373.5	330.0	328.4	323.8	341.3	369.4	365.4	433.8	410.0	384.1	332.8	347.1
45	311.4	341.9	371.2	321.4	328.7	317.3	345.7	360.0	377.2	334.5	312.5	378.9	336.9	339.0
46	308.9	345.2	373.0	327.0	323.3	330.0	346.2	365.0	370.0	438.9	411.5	384.5	332.4	345.0
47	308.9	346.9	376.3	322.4	327.5	325.8	349.1	366.0	375.9	435.8	421.6	388.0	340.0	350.5
48	317.3	348.3	369.1	329.7	329.7	333.7	339.3	366.0	364.5	436.3	408.2	393.8	335.5	349.0
49	319.0	347.0	372.5	327.3	332.7	330.8	351.9	367.9	367.9	430.3	403.0	388.2	345.0	346.1
50	300.1	342.8	360.0	319.2	314.1	318.4	325.5	363.3	348.5	433.9	396.5	399.2	340.7	343.8
51	318.0	348.1	378.1	324.7	335.5	330.0	340.0	363.0	368.3	431.0	402.5	393.6	339.7	343.9
52	318.0	350.0	375.4	333.1	339.7	336.5	339.8	366.9	360.0	430.7	394.0	387.9	336.4	350.0
53	322.5	346.0	369.3	327.8	336.4	329.0	347.9	365.4	375.4	425.0	394.0	390.0	340.5	346.1

7 Footnotes

[1] An avidity score is calculated by taking total intake during the test day and subtracting their water intake during the two-bottle test from this value. Then this difference is divided by the body weight of the animal and multiplied by 100 to make the score a percentage of the rats' body weight.

[2] Preference scores were calculated by taking the amount of saccharin consumed during the two-bottle preference test and then dividing that number by the total amount of fluid consumed during that test (water intake + saccharin intake).