Conditioned Stress-Eating and Stress Non-Eating in Rats, and their Preference for Food Sweetened with Sucralose

by

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Abstract

The current study examined different learning histories in relation to stress and food intake. In other words, stress-induced eating and non-eating could be due to different learned associations between a stressor and food. Seventeen male Sprague-Dawley rats were used to create a model of stress-eating and non-eating using operant conditioning. This model was then used to examine subjects' food intake and preferences for food formulas sweetened with three different amounts of Splenda: 0%, 10%, and 60%. These formulas were first presented to the rats individually (one-choice test) while a high-frequency tone (the stressor) was present and absent. The second test (two-choice test) exposed the rats to 2/3 formulas at the same time while the stressor was present and absent. It was found that all rats, regardless of group assignment preferred chows containing no Splenda, as demonstrated by an increase in food intake. This did not change as a factor of stress. Additionally, conditioned stress-eaters increased their food overall food intake when the stressor was present, as compared to when it was absent. This was not observed for conditioned stress non-eaters. Limitations, implications, and future directions are discussed.

Keywords: Operant Conditioning; Stress; Palatability; Food Intake; Sucralose; Preference

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Stress is highly prevalent amongst the Canadian adult population. Approximately 22.3% of Canadians over the age of 15 report experiencing a high degree of stress daily (Canadian Community Health Survey, 2008). Stress triggers a cascade of events within the body to prepare an organism to act in the face of danger. Stress can affect eating and digestion, and has been shown to have a bidirectional effect on food intake in both humans and animals. In other words, food intake has the potential to increase or decrease in response to chronic or acute stress. Typically, in animal research, stress causes a decrease in eating (Calvez, Fromentin, Nadkarni, Darcel, Even, Tome, Ballet, & Chaumontet, 2011; Marti, Marti, & Armario, 1994). However, increases in eating have also been observed especially when palatable foods were present (Adam & Epel, 2007; Pecoraro, Reyes, Gomez, Bhargava, & Dallman, 2004). Stress-induced eating of palatable foods falls under the category of hedonic hunger. Hedonic hunger is the desire to eat without there being a deficit in energy and often involves palatable foods (Lowe & Butryn, 2007). It has been demonstrated that palatable foods can trigger the reward system and depress the activity of the hypothalamic-pituitary-adrenal (HPA) axis, thus decreasing the effects of stress (Adam & Epel, 2007). This could be a reason why an increase in intake of palatable foods is sometimes produced in response to stress.

Stress and eating are very complex processes and involve various mechanisms throughout the body and brain. As such, it is still unclear as to which factors contribute the divide between stress-eaters and stress non-eaters (Greeno & Wing, 1994). Many factors have been proposed to contribute to the divergence of stress-eaters and stress non-eaters including but not limited to: individual differences (ex. weight), environmental factors (ex. palatability), and/or different learning histories. A previous study by Johnson and Emond (2017) looked at how different learning histories affected food intake during times of stress. The results suggested that

the two populations could be produced by different learned associations between a stressor and eating, brought upon by operant conditioning (Johnson & Emond, 2017).

The current study created animal models of stress eating and non-eating using operant conditioning. More specifically, stress-eaters were conditioned using negative reinforcement training, whereby eating would result in the removal of a stressor. The stress non-eating group was conditioned using punishment training, whereby eating would result in the presentation of a stressor. This model was then used to examine each groups' preferences for chows containing different levels of sweetener, while a stressor was present and when it was absent. The results of this study could provide further support for why these two populations of stress-eaters exist, and how the divergence occurs, thus deepening current understandings of stress-induced eating/non-eating, and food choice. Additionally, this study could give us insight as to how these two populations change their food intake in the presence of palatable foods, when exposed to a stressor.

Stress

In its most basic form, stress is described as state of threatened homeostasis within an organism's body (Johnson, Kamilaris, Chrousos, & Gold, 1992). Homeostasis an organism's internal equilibrium that is maintained by means of various physiological mechanisms and feedback loops (regardless of external environment). For example, in mammals, homeostasis keeps a stable internal body temperature, regardless of the temperature in the external environment. Shivering when it is too cold or sweating when it is too hot, are some examples of the mechanisms involved in maintaining a mammal's internal body temperature. Homeostasis is crucial for keeping an organism's body functioning at optimal levels (Chrousos & Gold, 1992). Stress disturbs this equilibrium, thus the stress response (how an organism responds to a stressful

situation) involves triggering behavioural and physiological changes in the body, in an effort to bring the body's internal environment back to equilibrium. Stress depends upon three main components: a) a stressor, which is any stimuli that threatens an organism's homeostatic state, b) a threatened homeostatic state, which is the stress an organism is experiencing, and lastly, c) an adaptive response which is what an organism does (physiologically and behaviourally) in an attempt to return to homeostasis (Johnson et al., 1992). Stress can cause extreme physiological, psychological, and behavioural changes to prepare an organism to cope with a threat. These changes have been linked to a multitude of health issues such as cardiovascular disease, high cholesterol, high blood pressure, obesity, diabetes, headaches, stomach issues, anxiety, depression, decreased immunity, and cancer (Cohen, Janicki-Deverts, & Miller, 2007). In addition, some individuals may develop unhealthy habits such as drug and alcohol abuse, or maladaptive eating, for example (Carlson, Buskist, Enzle & Heth, 2005).

When an organism is faced with a stressful situation, it triggers a cascade of events within the brain and body. It starts with the activation of the hypothalamus pituitary adrenal (HPA) axis, specifically the paraventricular nucleus (PVN) area of the hypothalamus, and the sympathetic nervous system (Adam & Eple, 2007; Dedovic, Duchesn, Andrews, Engert, & Pruessner, 2009). Cells within the PVN release corticotropin-releasing hormone (CRH), which then circulates towards the pituitary gland, and stimulates the release of adrenocorticotropic hormone (ACTH) (Dedovic, et. al., 2009). ACTH travels towards the adrenal cortex and triggers the release of cortisol (or corticosterone in rodents) in the blood (Dedovic, et. al., 2009). By binding to receptors in the limbic system portion of the brain, cortisol actually regulates its own production by means of a negative feedback loop (Adam & Eple, 2007; Dedovic, et. al., 2009). Thus, when cortisol is released, it also sends signals to the brain to stop its production (Adam & Eple, 2007;

Dedovic, et. al., 2009). Typically, when the stressful situation has ended, cortisol will serve as a signal to the brain to terminate the physiological stress response within the organism, so that the body can return to homeostasis (Adam & Eple, 2007). However, the duration of this process is dependent upon the severity and duration of the stressful event (Johnson et al., 1992).

This negative feedback loop which results in cortisol terminating the body's stress response is important since long-term exposure to this hormone can have harmful effects on the brain and body. It has been shown to cause a decrease in bone density because it inhibits bone formation, which can lead to osteoporosis (McEwen, 1998). Cortisol can damage the hippocampus causing memory and cognitive impairments (McEwen, 1998). It can also suppress immune system activity, making individuals more vulnerable to disease (Segerstorm & Miller, 2004). Cortisol increases blood pressure, and contributes to cardiovascular disease (Chrousos & Gold, 1998). As such, this cortisol negative feedback loop that was discussed earlier, is important for maintaining the organism's health and well-being (Adam & Eple, 2007). The body works to reduce the stress response in order to return to a state of homeostasis, which may then motivate an organism to behave in a way that is favourable for this process.

As described previously, the stress response is dependent upon the duration of the stressful event. That being said, stress is categorized into two types: acute and chronic. Acute stress is defined as being short in duration, and is triggered by a single or brief exposure to a stressor. Examples of acute stress in humans include going for a work interview, or having to slam on the breaks while driving to avoid a collision, to name a few. In animal models, such as the current study, examples of acute stress include foot shocks, brief restraint, or the presentation of a loud auditory stimuli (Maniam & Morris, 2012). Stress that is long-term is described as chronic stress. Chronic stress is triggered by repeated or prolonged exposures to a stressor over a

long period of time (Maniam & Morris, 2012). An example, of chronic stress in humans could be a demanding environment such as university, where a person is consistently exposed to heavy work-loads, with tight deadlines to meet. An example of chronic stress in animal research could be having the animal restrained for many hours at a time or restraining the animal day after day, for prolonged periods of time each day (Maniam & Morris, 2012).

Acute stress is typically more manageable and less harmful to an organism as compared to chronic stress because it eventually leads to the deactivation of the HPA-axis through the negative feedback loop discussed above, when the threatening situation ceases (Maniam & Morris, 2012). In fact, acute stress has even been shown in research to have bidirectional effects on task performance (LeBlanc, 2009; LeBlanc, Woodrow, Sidhu, & Dubrowski, 2008). This is not the case for chronic stress, where glucocorticoid secretion is long-lasting, which then keeps the HPA-axis activated (Johnson et al., 1992). This results in cortisol remaining in circulation within the body for extended periods of time, which can lead to deterioration of an organism's health (McEwen, 1998). In light of this information, it is important to acknowledge that the present study exposed its subjects to acute stress, which will be discussed in more detail in the methodology.

In addition to different types of stress, there are also different types of stressors. Stressors, which are anything that causes stress for an organism, can be physical or psychological. A physical stressor is a stimulus or situation that can cause bodily injury. An example would be a confrontation with a wild animal or extreme weather (Dickerson & Kemeny, 2004). On the other hand, psychological stressors negatively affect emotions; they induce frustration, anxiety and fear (Johnson et al., 1992). An example would be having to give a presentation in front of a large audience. The current study used a high-frequency tone as its

stressor. This is because noise stress has been shown in past research to be a reliable aversive stimulus that can produce a stress response and other behavioural changes in lab animals (Chandralekha, Jeganathan, Viswanathan, & Charan, 2005; Johnson & Emond, 2017; Krebs, Macht, Weyers, Weijers, & Janke, 1996; Monjan & Collector, 1977; Weingarten, 1985). Noise stress affects lab animals both physiologically and behaviourly. A study by Chandralekha et al. (2005) found that exposing rats to 120dB noise stress effectively triggered the release of corticosterone, in both acute and chronic stress conditions. As mentioned previously, corticosterone is a key indicator of stress in rodents (cortisol for humans). In addition, a study by Krebs et al. (1996) examined the effects of noise stress in rats on other behaviours such as eating, grooming, exploring, and defecation. Their stressor (white noise) was set at 95dB and they found that rats increased their grooming and exploring behaviours in the test chamber. This stressor was also effective in producing changes in eating behaviour. More specifically, the rats decreased the duration of the time spent eating, but increased their rate of eating (Krebs et al., 1996). Noise stress falls within the category of psychological stressors, since it does not cause bodily harm (Campos, Fogaca, Aguiar, & Guimaras, 2013). This information pertains to the current study because psychological stressors have shown to be the ones that most often alter feeding behaviour in humans and in animals (Campos et al., 2013).

It is clear now that stress produces notable changes within the body and brain. Therefore, it can be suggested that an organism's behaviour, including feeding, can be affected by stress. Some of these behavioural changes, such as changes in food intake, may help the organism react and cope with a stressful situation (Chrousos & Gold, 1992).

Stress and Eating

The classic stress response prepares the body for fight or flight. To increase the chances of survival, energy must be allocated to parts of the body that play a key role in this fight or flight response (Chrousos & Gold, 1992). This means the activation of other processes, such as digestion, is suppressed (since it is less important). This typically produces a reduction in hunger and feeding behaviour (Chrousos & Gold, 1992). However, studies have found that stress influences eating behaviour in a bidirectional manner (Adam and Epel, 2007). Through self-report measures, Wallis and Hetherington (2009) demonstrated that of their 89 participants, when experiencing stress, 48% reported eating less food, while 43% reported eating more food, and the remaining 9% reported that they did not change their eating habits, as compared to their normal food intake. Wallis and Hetherington (2009) ended up excluding participants who reported no change in their food intake during stress and found that there were just as many self-declared stress-eaters as there were stress non-eaters.

This bidirectional stress-eating trend has also been observed amongst animals. However, research has shown animals generally reduce their food intake when they are stressed. A study by Marti et al. (1994) tested three different types of stressors (immobilization, restraint, and handling) and their effects on eating behaviour in rats. Immobilization was induced by taping the animal's forelimbs to a wooden board, and by restricting head movements with two metal loops around the neck. Restraint was introduced by placing the animal in a snug plastic tube. The third stressor was handling, where a researcher would hold the animal for one minute. They found that immobilization, which was the most severe of the stressors, not only caused the most stress but significantly reduced the rats' food intake and body weight. Marti et al. (1994) explained that the

observed decrease in food intake was likely due to the release of CRF (released during stress) which has been shown in the past to have anorectic effects.

Similarly, Calvez et al. (2011) used both restraint and forced swimming to induce mild, acute stress on rats and observed their effects on food intake. Calvez et al. (2011) found that both stressors produced a significant decrease in food intake. Restraint caused long lasting decreases in food intake, which then reduced the animals' weight gains. Calvez et al. (2011) noticed that the rats were spending more time grooming which was competing with eating behaviour. They believed that this was due to the expression of POMC in the brain, which is responsible for satiation effects and grooming behaviour.

While this and other research has shown how stress decreases food intake in animals, some research has demonstrated that stress can produce the opposite outcome, and instead, increase feeding behaviour in animals. Studies using the mild tail pinch stress model, have consistently produced an increase in eating behaviour in rats (Antelman & Szechtman, 1975; Hawkins, Cubic, Baumeister, & Barton, 1992; Levine & Morley, 1981). Ely, Dapper, V, Marasca, Correa, Gamaro, Xavie, Michalowski, Catelli, Rosat, Ferreira, & Dalmaz, (1997) used restraint as their stressor, and found that rats increased their intake of sweetened food when under stress. Many researchers have tried to explain why both humans and animals respond to stress either by increasing or decreasing their food consumption, and what factors contribute to these distinct changes in behaviour. In humans, researchers have proposed an individual-differences model, which was first introduced to compare stress-eating behaviour between obese and normal weight individuals (Greeno & Wing, 1994). In general, the individual-difference model suggests that stress-induced changed in eating behaviour may be due to individuals differing in weight (obese vs. normal), eating style (restrained vs. unrestrained), and/or gender

(Greeno & Wing, 1994). However, these results are mixed and suggest that these individual differences do not accurately predict stress-induced eating or under-eating (Greeno & Wing, 1994). It was proposed that perhaps different learning histories between individuals could help explain these stress-induced food intake changes. In other words, individuals could be associating the feeling of stress and their feeding response, differently from one another. A study by Johnson and Emond (2017) tested this hypothesis, and found that stress-eating and stress under-eating could successfully be trained in rats. On that note, this study focused on stress-induced eating and non-eating as two distinct learned behaviours, in response to stress.

As discussed previously, stress can alter an organism's eating behaviour (Dallman, 2010; Wallis & Hetherington, 2009). Both intake (less, more, no change) and food choice can be affected (Dallman, 2010; Wallis & Hetherington, 2009). When experiencing stress, individuals report eating more palatable and calorie-dense foods (Adam & Epel, 2007; Dallman, 2010; Wallis & Hetheringron, 2009). There seems to be a shift towards food that would normally be avoided for weight-loss or health concerns. With that said, it seems as if the hedonic properties of food play an important role within the scope of stress and food intake. Another factor that may be contributing to the bidirectional stress-induced eating trend observed in humans and animals, is palatability.

Palatability

Palatability is the hedonic property of food or beverages (Lowe & Butryn, 2007), and is an important aspect of determining what and how much we eat (Yeomans, 1998). It has also been described as an organism's preference for one food or beverage, over the other (Young, 1966), which is an key factor within the scope of this study. In general, it has been suggested that the more palatable a food or solution is, the better it tastes to an organism. In fact, animal

research has demonstrated that when given a choice in their normal state, rats prefer solutions that are sweeter as compared to blander flavours (Berridge, 1996; Levi, Limebeer, Ferdinand, Shillingford, Parker, & Leri, 2014; Scalfani & Nissenbaum, 1987; Young, 1966). A study by Scalfani and Nissenbaum (1987) compared various sugar solutions (polycose, maltose, and sucrose) to water, using a 24hr 2-bottle taste test. Overall, rats consistently preferred the sugar solutions over water, even though the sugar concentrations were relatively low.

Highly palatable foods tend to have elevated concentrations of fat, sugar, and/or salt, and usually contain large amounts of calories. An evolutionary explanation as to why animals, like rats, find sweeter foods more palatable is because a food's sweetness is an indicator of a food's caloric content (Gearhardt, Corbin, & Brownell, 2009). More calories provide the organism with more energy and increases its chances of survival (Gearhardt et al., 2009). For this reason, sweeter foods are more rewarding to the organism because of their association with calories.

When referring to palatability and eating, it is important to note that hunger can be homeostatic or hedonic. Homeostatic hunger is triggered by the body's need for energy to maintain homeostasis (Lowe & Butryn, 2007). Hedonic hunger, which is most relevant when discussing palatability, is attributed to eating even when having been satiated by blander foods (Lowe & Butryn, 2007). This type of hunger is dependent upon the palatability of food and its availability in the immediate environment (Lowe & Butryn, 2007). An example of hedonic hunger would be having dessert after a very large meal, the dessert is consumed for the rewarding properties of its pleasant taste, and not for the calories it provides (Lowe & Butryn, 2007).

Palatability is an orosensory cue in food which, as mentioned previously, affects food intake in a bidirectional manner. Highly palatable foods such as those high in fat and/or sugar

contents, typically trigger an increase in food intake. It was proposed by Le Magnen (1985) that these foods can induce an "appetite effect". The appetite effect is the increase in appetite as a result of eating (Le Magnen, 1985). Thus, rather than bringing the organism closer to satiation, ingesting highly palatable foods makes them hungrier, and increases their food intake. On the other hand, some flavours such as bitter and sour, are considered as less palatable to humans and animals (Nisbett, 1968; Yeomans, 1998) and decrease food intake (Nisbett, 1968). Then, there are some flavours such as salt that can both increase and decrease palatability of food depending on its concentration (Yeomans, 1998). This bidirectional effect on palatability has also been observed with sweet flavours, despite being a generally liked flavour even at high concentrations (Monneuse, Bellisle, & Louis-Sylvestre, 1991). For example, adding too little sugar to a coffee can make it taste too bitter, while adding too much sugar may make it too sweet and undesirable. The present study will be manipulating palatability by adding different amounts of a non-caloric sweetener to rat chow mixtures. Highly palatable foods tend to contain high concentrations of sugar, and this flavour is generally liked by both humans (Berridge, 1996) and animals (Berridge, 1996; Levi et al., 2014; Young, 1966)

Palatability of food can not only be increased or decreased by changing concentrations of a certain flavour, but also by repeated exposure/ experience (Colangelo, Levi, & Leri, 2014; Pliner, 1982; Zajonc, 1968), appetite (Yeomans, 1998), and emotional state (Dallman, 2010). This study focused on emotional state, specifically on how stress interacts with palatability.

Palatability and Stress

Stress, eating, and the palatability of food all interact with the brain's reward system. This interaction is largely observed with endogenous opioids. There is evidence that when the HPA-axis is activated as a result of stress, endogenous opioids are also being released (O'Hare,

Shaw, Tierney, Levine, & Shepard, 2004). This is because endogenous opioids act as an HPA-axis depressant, and protect the organism against the negative effects of stress (Drolet, Dumont, Gosselin, Kinkead, Laforest, & Trottier, 2001). There is increasing evidence that the ingestion of palatable foods stimulates the release of endogenous opioids. As such, these foods decrease HPA-axis activity which then terminates the stress response (Kreek & Koob, 1998). In addition, it has been shown that when an opioid antagonist is injected into the substantia nigra (part of the reward system) portion of the brain, it significantly attenuated stress-induced eating in rats (Hawkins et al., 1992).

Dopamine also seems to also play a role in stress, eating and palatability. Some foods, such as sugar and lab chow can stimulate the release of dopamine (Aveena, Rada, & Hoebel, 2008). This release then puts a reward value on the food and can reinforce the eating behaviour (Aveena et al., 2008). Stress can decrease the reward system sensitivity to reward (Born, Lemmens, Rutters, Nieuwenhuizen, Formisano, Goebel & Westerterp-Plantenga, 2010). Therefore, in theory, food with increased palatability (ex. increased amounts of a liked flavour) should be needed to trigger the same sense of pleasantness experienced when it is consumed under normal circumstances.

As discussed earlier, under normal circumstances, animals generally prefer sweeter flavours as compared to bland ones. However, when under stress, this preference for sweeter flavours seem to fluctuate, and results vary between studies. Sampson, Muscat, Phillips, & Willner, (1992) conducted a study where they exposed rats to various mild stressors such as food and water deprivation, overnight illumination, cage tilt, paired housing, soiled bedding, white noise, and stroboscopic illumination for a period of 10 weeks. During this time, the animals were presented with a wet-mash (powdered chow mixed with water) where its sucrose concentration

was increased on a weekly basis, to a total of 40%. They found that stressed rats appeared to be insensitive to changes in sucrose concentration. Food intake and rate of eating were similar to that of mash with lower sucrose concentrations. The researchers suggested that this failure to increase their food intake could be interpreted as a decrease in the rewarding properties of sweet food (Sampson et al., 1992). Supporting the idea that stressed rats are insensitive to palatability changes, is a study by Matthews, Forbes, & Reid (1995). They found that mildly stressed rats did not show a preference for a 0.9% sucrose solution as compared to control animals. It is important to note, however, that this sucrose concentration was very low and that may be why differences were not observed in this study.

On the contrary, Pecoraro et. al. (2004) found that when rats were chronically stressed by means of restraint, they increased their intake of lard and sucrose, as compared to regular lab chow. In turn, they observed a decrease in the stress response. The researchers believed that this was because stress has been shown to increase opioid signalling within the brain, which seems to encourage the consumption of palatable foods (Pecoraro et. al., 2004). They also suggested the general accumulation of white adipose tissue brought upon by the increased intake of sucrose and lard, triggered an increase in leptin within the body, which decreased signalling of neuroendocrine motor neurons (Pecoraro et al., 2004). The results from Pecoraro et al. (2004) are in line with the hypothesis that eating palatable foods during stress is likely an adaptive coping mechanism.

This hypothesis was also supported in human research. Zellner, Loaiza, Gonzalez, Pita, Morales, Pecora, and Wolf (2005) gave their participants a choice between grapes, peanuts, chips and chocolates. They found that stressed participants ate more chocolate, as compared to chips, peanuts, and grapes, while non-stressed participants ate more grapes as compared to the other 3

food choices. While both grapes and chocolate are sweet in taste, chocolate is rated as more palatable than grapes (and also considered less healthy). In addition, chocolate contains more fat, sugar, and salt (components of highly palatable foods) as compared to grapes. This study highlighted the fact that when stressed, there is a shift towards highly palatable foods, as compared to blander (healthier) foods. Thus, if an individual often finds themselves feeling stressed, and is continuously increasing their intake of "snack foods", it could lead to more severe health concerns, such as obesity.

The studies above looked at food preferences by presenting their subjects with a varied selection of food, and measured their intake. This is one of many ways to measure palatability, and food choice in research. A common method used in animal research, is the one (or more) bottle taste test. This test inserts flavoured solutions (ex. sweet or bitter) in bottles with sippers, and then places them in the animal's home cage. The animal can then drink the solutions freely, and after a select period of time has elapsed, the researcher can remove the bottle(s) and measure how much of the solution was consumed. This can be done with a single bottle, and up to six bottles at one time (Tordoff & Bachmanov, 2003). However, the most popular method is the two-bottle taste test (Tordoff & Bachmanov, 2003). The one-bottle test does not allow for a direct comparison in preference between two or more solutions because it only lets the animal ingest one solution at a time. However, this method allows the researcher to measure intake of a solution, without the influence of another solution. It is a good method to look at overall consumption of a specific food or solution. In a non-research setting, humans are likely to have access to more than one palatable food in their environment at any given time.

Many studies on taste preference use the two-bottle taste test to compare an animal's preference for two solutions chosen by the researcher (Tordoff & Bachmanov, 2003). This

technique allows the animal to choose which solution it would prefer to consume. The current study modified this technique by testing with wet-mash (powder/water) rather than liquid solutions. Three different amounts of a non-caloric sweetener were then added to the wet-mash. Two tests were performed: a one-choice test, where rats had access to one of the three chows at a time, through a single bowl placed directly in an operant chamber. The second was a two-choice test, where rats had access to a combination of 2 of the 3 chows at the same time.

Overall, it is clear that stress interacts with eating behaviour by either causing an increase or a decrease in food intake through various mechanisms within the body and brain. However, the results of those studies examining humans' and animals' preferences for palatable foods during stress are mixed. Past studies have not taken into consideration the increased calories that comes with sweetening food with sucrose (table sugar) when looking at palatability. The current study used Splenda to alter the palatability of food. Splenda is a sucralose-based, non-caloric sweetener, which means it changes the taste of food without impacting the calorie content. Splenda is a well-known product that sells itself on having the same sweetness ratio as table sugar, without the added calories. In other words, one cup of Splenda is equal to one cup of table sugar. Furthermore, past studies have not looked at manipulating the palatability of their tasteants specifically using Splenda, to examine their subjects' preferences when stressed, and when in a normal state. This study aimed to fill these gaps in research, by comparing stressed and non-stressed rats' preferences for three concentrations of Splenda. This allowed for direct observations of possible interactions between stress and the hedonic properties of food, amongst conditioned stress-eating and conditioned stress non-eating rats, when they were stressed and non-stressed.

While palatability is an environmental factor that may play a role on food preferences while animals are stressed, the present study wanted to consider a developmental variable that could affect stress-related eating responses. More specifically, the current study examined if prior learning and associations between stress and food intake could have a subsequent effect on stress-related eating responses.

Operant Conditioning/ Operant Model of Learning

There have been many studies examining the bidirectional trend of stress eating, that has been observed in both humans and animals. However, there has not been a comprehensive theory put forward of how this divergence occurs. One possible theory is that the divergence is produced through operant learning when organisms experience different contingencies between the response of food intake and the outcome of stress. For example, if an organism eats food in response to stress (a negative stimulus), and it causes a reduction in the stress response, then stress-eating will be reinforced. On the other hand, if food intake during stress has negative consequences then stress non-eating will be reinforced.

Operant conditioning is composed of 3 main components: a behaviour of interest (response), a stimulus (positive or negative) and a contingency between the stimulus and the response (Skinner, 1938). This type of learning shapes behaviour (increases or decreases its frequency) by means of presenting or removing a stimulus (Skinner, 1938). The stimulus can be a reinforcer or a punisher (Skinner, 1938). A stimulus that reinforces a behaviour causes that behaviour to increase in frequency, while one that punishes causes a behaviour to decrease in frequency (Skinner, 1938). There are 4 different ways to shape behaviour using operant conditioning: positive reinforcement, negative reinforcement, punishment training, and omission training. The present study used negative reinforcement and punishment training to shape an

animal's eating behaviour in response to stress. Negative reinforcement involves the use of an aversive stimulus to increase the probability of a desired response. When a desired behaviour is performed, the aversive stimulus is removed. As long as the behaviour is maintained, the subject will not be exposed to the aversive stimulus. The frequency of the desired behaviour increases because it becomes associated with the removal of an aversive stimulus. Punishment training also uses an aversive stimulus to manipulate the frequency of a behaviour. Unlike negative reinforcement, this type of contingency is used to decrease the frequency of a behaviour. When a behaviour is performed, the subject is exposed to the aversive stimulus. The subject learns that their behaviour is associated with the presentation of something unpleasant.

Learning influences many behaviours including feeding. Feeding is usually initiated by hunger or when the organism experiences a deficit in energy. Early studies in this field explored classical conditioning and its involvement in feeding. Grant and Milgram (1973) showed that rats could make a strong association between a certain environment and feeding. In their study, rats were food-deprived and given the opportunity to eat within a distinct environment. The rats learned that they would be given food when placed in that environment. In fact, rats initiated feeding within the training environment, even when they were satiated.

A study by Weingarten (1983) demonstrated that rats could be trained to associate eating with a cue of choice. After a few conditioning trials, the rats developed strong, persistent responding to the cue. Even when the rats were satiated, they continued to initiate feeding in the presence of the cue and independently of their caloric needs. There is a handful of evidence showing that operant conditioning can influence meal initiation, however it can also inhibit feeding.

A recent study by Johnson and Emond (2017) successfully demonstrated that learned associations between food and an aversive stimulus can either trigger feeding or inhibit it. This shaped two distinct groups of rats: those who fed and those who refrained from eating in the presence of an aversive stimulus. This behaviour was generalized to a variety of aversive stimuli such restraint, a foot-shock, and a high-frequency tone, which is the stressor chosen for the present study (Johnson & Emond, 2017). Negative reinforcement and punishment training can therefore be used to simulate or reduce feeding behaviour, in response to stress.

Current Study

The present study was composed of two parts. The first part used operant conditioning to shape feeding behaviour in rats, in response to stress. The goal was to create an animal model of each of the two distinct populations of stress eaters observed in humans: one group that increases their food intake, and another that decreases their food intake, when experiencing stress. It has been consistently demonstrated that humans and occasionally animals respond to stress by either under-eating or over-eating, however it is still unclear as to what causes this divergence. It was anticipated that this study would provide further evidence that different learning histories contribute to the emergence of two distinct population of stress eaters.

The second component looked at the role of palatability as another possible factor contributing to the bidirectional stress-induced eating trend observed in humans, while employing the animal models created in the first portion of this study. This was accomplished by crafting 3 wet-mash mixtures made up of 3 different Splenda concentrations: 0%, 10%, and 60%. These wet-mashes were presented to the subjects either individually by means of the one-choice test, or in varying combinations of 2 of the 3 mixtures through the two-choice test. These tests were performed while a high-frequency tone (the stressor) was present, and also when it was

absent. These particular Splenda concentrations were chosen because a study by Levi, et. al. (2014) demonstrated, while comparing various concentrations of high-fructose corn syrup (HFCS) solutions, that self-administration response rate was similar for that of 25% and 50%. Significant differences in response rate emerged between the low concentration solution of HFCS (8%) and the higher concentrations of HFCS solutions (25% and 50%). Therefore, it was important that there be a large enough difference between the concentrations, for the animal to notice a difference in sweetness levels. Splenda (sucralose), is a non-caloric sweetener which allowed for changes in sweetness levels, without increasing the calorie content of the chow mixtures. This was done to remove the typical rewarding properties of calories found in more palatable foods.

Predictions

Training and Verification Test. With successful training, it was expected that the Conditioned Stress-Eating (CSE) group would increase their food intake, while the Conditioned Stress Non-Eating (CNE) group would decrease their food intake when the stressor is present as compared to when the stressor is absent. These predictions were made because following training, CSEs should associate their feeding response with the removal of the stressor, while CNEs should associate their feeding response with the presentation of the stressor. When the stressor is absent, their feeding would return to each groups' baseline.

Experiment 1: One-Choice. Similar to the predictions in training/verification test, it was hypothesized that the CSE group would increase their overall food intake of all three chows (0%, 10%, and 60%) while the CNE group would decrease their overall food intake when the stressor is present. This is due to their learned associations with the stressor.

Furthermore, it was hypothesized that both groups would prefer the sweeter chows (10% and 60%) over the bland chow (0%) under normal circumstances (non-stressed). This is because it has been shown in research that when given a choice, under normal circumstances, rats prefer sweet solutions (Yeomans, 1998; Young, 1966). In particular, it was predicted that both groups would show a preference for the 10%-Splenda chow when the stressor was absent. This is because in a self-administration study by (Levi, et al., 2014), which was conducted with no stressor and with high-fructose corn syrup (HFCS), demonstrated that response rates were the highest for 25% HFCS as compared to 8% HFCS, and 50% HFCS. Thus, rats preferred the "middle" concentration.

Lastly, it was hypothesized that there would be a stress-induced preference for the 60%-Splenda chow, especially for CSEs. This is because it has been shown that stress can decrease the rewarding properties of palatable food, thus in theory, palatability should be increased to trigger the same rewarding effects (Born et al., 2010). CSEs should be more sensitive to this palatability shift, since they would have learned that feeding results in the removal of a stressor. Additionally, the positive effects of ingesting palatable foods during stress, should be amplified with 60%-Splenda chow.

Experiment 2: Two-Choice. To start, it was hypothesized that when the stressor is present, CSEs would increase their overall intake of all three chows (0%, 10%, and 60%), while CNEs would decrease their overall intake of all three chows (0%, 10%, and 60%) when compared to their intake when the stressor is not present.

As for their preference of palatable foods, it was hypothesized that there would be a stress-induced preference for chows containing higher concentrations of Splenda, in each of the three combinations. In combination 1, it was expected that subjects would prefer 10%-Splenda mix

more than 0%-Splenda mix. In combination 2, it was expected that subjects would prefer the 60%-Splenda mix over the 0%-Splenda mix. Finally, for the third combination, it was expected that subjects would prefer the 60%-Splenda mix over the 10%-Splenda mix. Similar to experiment 1, it was hypothesized that this stress-induced preference would be especially noticeable within the CSE group. This is because this group should have learned to associate feeding with the removal of a stressor, and as mentioned previously, the consumption of palatable foods has been shown to decrease the stress response in humans and animals (Pecoraro et al., 2004). The release of endogenous opioids brought upon by the ingestion palatable food decreases the activity of the HPA-axis, and helps terminate stress response (Pecoraro et al., 2004). Furthermore, as discussed earlier, stress has been shown to decrease the rewarding properties of palatable foods, thus a more intense flavour should be required to produce a decrease in the stress response. Finally, similar to experiment one, when the stressor is absent, it was hypothesized that there would be a unanimous preference for the 10%-Splenda mix (in combination 1 and 3), and for 0%-Splenda (in combination 2).

By looking at the effects of stress and palatability of rats trained to associate stress with positive or negative outcomes, the results of this study will hopefully provide additional support as to why two distinct populations of stress-eaters exist, and how this divergence occurs, by demonstrating that these changes in feeding behaviour could be due to different learned associations between a stressor and feeding. Additionally, it could provide a better understanding of the role of palatability as a possible factor contributing to stress-induced eating and under-eating, as well as how these two populations react to changes in their food's sweetness concentrations, under stress.

Methodology

Subjects

This study used male Sprague-Dawley rats (n=17) weighing between 100-125g at the beginning of the experiments. Rats were obtained from Charles River Laboratories (Montreal, QC) and singly housed in plastic cages. They were maintained on a 12h light/dark cycle (7am ON, 7pm OFF). Rats had access to *ad libitum* water and chow in their home cage, except during behavioural training and testing. The experiments were approved by the Animal Care Committee of Laurentian University and were performed to the standards of the Canadian Council on Animal Care (see Appendix).

Materials

All training and testing sessions took place in a single operant chamber measuring 30cm x 20cm x 25cm, which was customized to meet experimental needs. The operant chamber was equipped with a pellet dispenser, a metal grid floor, and a house light. The house light was controlled manually with a switch on the side of the chamber. The operant chamber was wrapped with black Bristol board except for one panel where observations could take place. This was done to minimize visual distractions for the animal. Holes or gaps in the chamber were sealed using black electric tape. The precision pellets and the Splenda wet-mashes were placed directly in the chamber, in one or two bowls (depending on the experiment). The bowls were secured to the operant chamber with Velcro. This prevented the animals from spilling the content of the bowls.

In their home cages, rats were given a standard lab chow. Unsweetened precision pellets (45mg) by TestLab, were used during behavioural training sessions. The palatable (and neutral) foods were presented to the subjects in a wet-mash. The mash was prepared by first grinding the

precision pellets used for training, into a fine powder. Splenda (sucralose) was added to the powder to create a 10%-Splenda or a 60%-Splenda mixture. Splenda was used because it is a well-known non-caloric sweetener, which allowed for the mixtures to be sweetened without increasing the calorie content. Water was then added to the mixtures, and stirred until the mash became homogenous, with the consistency of baby food. The 0%-Splenda wet-mash was composed of 10g of pellet powder and 5ml of water, the 10%-Splenda wet-mash was composed of 9g of pellet powder, 1g of Splenda, and 5ml of water, and the 60%-Splenda wet-mash was composed of 4g of pellet powder, 6g of Splenda, and 5ml of water.

Habituation

Prior to the start of the behavioural training, the animals were given four days to familiarize themselves to their home cages and colony room. Following these four days, the rats were each handled for 10 minutes per day, for five days. During the following three days, handling continued, and the animals' weights were recorded. This procedure continued for seven days with the addition of measuring and recording home-cage food consumption. After each rat was handled and weighed, home-cage food consumption was measured by placing 200g of lab chow in their feeder and weighing the remaining food 24hrs later. The subjects were then introduced to the operant conditioning box where they would be trained and tested. The animals were individually placed in the box for two minutes each. This was repeated for three days. The stressor was not present during this step.

To minimize neophobia during training and testing, the rats were introduced to the precision pellets in the familiarity of their home cage. They were each given 10 pellets and allowed 24hrs to consume them. The Splenda wet-mashes were introduced in the same manner as the precision pellets; the rats were given 24hrs to consume 2g of 60%-Splenda wet-mash, and

2g of 10%-Splenda wet-mash, four days before testing with these wet-mashes (2 exposures to each mash). By the morning, it was verified that all of the wet-mash had been consumed overnight. This was done following the completion of behavioural training. This was not done with the 0%-Splenda wet-mash because the rats were exposed to the neutral flavour during training.

The rats were randomly assigned to one of two experimental conditions: conditioned stress-eaters (CSE) or conditioned stress non-eaters (CNE). Random assignment was performed using an online random-assignment generator. Average weights and average food consumption were compared between groups to ensure that they were as similar as possible. Statistical analyses were conducted to verify that there were no statistically significant differences in weight or food consumption between the two groups prior to training.

Food Restriction

To facilitate learning during training, the rats were food restricted overnight (12hrs) before the beginning of each training session. The rats were also food restricted in the same manner prior to testing. The animals were food restricted in the same order that they would be trained or tested the next day. The rats were given 15g of chow (about two and a half blocks) during this restriction period, which was on average, half of what they consumed in 24hrs. This was to make sure that food deprivation was not endured for an excessive amount of time (i.e. more than 18hrs) for those animals training/testing later in the day. To ensure normal growth and development, the rats were given *ad libitum* access to regular chow immediately after training/testing and were given a rest day between training days and tests.

Training

All training sessions took place in an operant chamber. Each of the rats received 14 conditioning trials. The conditioning trials utilized a high frequency tone (98dB, 2184Hz) as the aversive stimulus. A high frequency tone is often used in research to produce stress in lab animals. It has been shown to be an effective stressor for conditioning (Chandralekha, et. al., 2005; Johnson & Emond, 2017; Krebs, et. al., 1996; Monjan & Collector, 1977; Weingarten, 1985). Before the start of each trial, the animal was placed in the operant chamber, the house light was manually turned on to indicate the start of the trials, and 20g of pellets were placed in a bowl directly in the chamber. Eating was defined as chewing the provided food for 20 seconds. At the end of each trial, the house light was manually turned off, the rat was removed from the chamber, and food consumption was measured. The operant chamber was cleaned between each animal to avoid scent contamination.

Conditioned Stress-Eaters (negative reinforcement training). The goal of this training was for the animal to associate eating with the removal of an aversive stimulus. This training would create our conditioned stress-eating (CSE) group. Nine of the 17 rats were randomly assigned to receive this training. All training trials lasted 18 minutes. The animals were placed in the operant chamber and were allowed one minute to orient themselves (no pellets, no house light). The house light was then manually turned on, and the bowl of pellets was placed in the chamber. The lid of the chamber was secured, and the high frequency tone was immediately turned on and maintained until the animal started eating. When the animal chewed the food for 20 seconds, the tone was turned off. If the animal stopped eating for 20 seconds (no chewing), the tone was turned on again. Thus, the tone was only present when the animal was not eating. With this training, it was predicted that rats in this group would learn to increase their food

intake when a stressor was present, since the response of eating was associated with the outcome of the removal of the stressor. The bowl of pellets was weighed every 6 minutes to measure food consumption over time.

Conditioned Stress Non-Eaters (punishment training). The goal of this training was for the animal to associate eating with the presentation of an aversive stimulus. This training would create our conditioned stress non-eating group (CNE). Eight of the 17 rats were randomly assigned to receive this training. Just like negative reinforcement training, these trials also lasted 18 minutes. Identical pre-trial steps were followed (orientation, house light, pellet bowl and chamber lid), except for the fact that the high-frequency tone was turned off at the beginning of the session. When the animal began eating the precision pellets for 20 seconds, the high frequency tone was turned on and maintained until the rat stopped chewing for 20 seconds. This process was repeated in accordance with the subject's eating behaviour. Thus, the tone was only present when the animal was eating. With this training, it was predicted that rats in this group would learn to decrease their food intake when a stressor was present, since the response of eating was associated with the outcome of the presentation of the stressor. The bowl of pellets was weighed every six minutes to measure food consumption over time.

Experiments

Verification Tests. Following the completion of the 14 training trials, two verification tests were conducted; one with the high frequency tone on, and another with the tone off. These tests were performed to determine whether or not training successfully manipulated the subjects' eating behaviour. One by one, subjects were placed in the operant chamber and allowed one minute to orient themselves. The house light was manually turned on, 20g of pellets in a bowl was placed in the chamber, and the lid was secured. The high frequency tone was immediately

turned on and maintained for the duration of the trial (10 minutes). If training was successful, the stress-eating group should consume significantly more pellets in the presence of the high frequency tone compared to when it is absent. On the other hand, stress non-eaters should consume significantly less pellets in the presence of the high frequency tone compared to when it is absent. Identical steps were followed for the second test except that the high frequency tone was absent. The expectation was that pellet consumption should be at baseline for both groups since subjects should not be stressed at this time.

Experiment 1: One-Choice. In this experiment, subjects were exposed to a single wet-mash mixture at a time. A one-choice test allows the researcher to measure of food intake without the influence of another food or solution present. It is a forced choice test as in, the animal can only choose to consume (or not consume) one type of food or solution.

Subjects were divided into two groups, consisting of an equal mix of CNE and CSE. The two groups were tested on alternating days to allow them to rest between tests. Additionally, the groups' testing order, testing condition, and wet-mash was counterbalanced to prevent order effects. All 17 rats were exposed to 0%, 10%, and 60%-Splenda wet-mashes individually, while the high frequency tone was present (stress condition) and also while it was absent (non-stress condition). Before the beginning of the trial, 15g (10g of powder and 5ml of water) of a select wet-mash was prepared and an initial weight was recorded, so that wet-mash consumption could be measured throughout the session. The bowl of mash was weighed at three, six, and 10 minutes. Behavioural measures (rearing and grooming) were recorded throughout these trials. All trials lasted 10 minutes. Similar to the training procedures, the rats were placed in the operant chamber and allowed one minute to orient themselves. Next, the house light was turned on, one bowl of wet-mash (either the 0%, 10%, and 60%-Splenda wet-mashes, depending on the trial)

was placed in the chamber, and the chamber lid was closed. When 10 minutes had elapsed, the house light was turned off to signal the end of a session. The rat was removed from the chamber and brought back to the colony room. The operant chamber was wiped down with a disinfectant spray to prevent scent contamination, and fresh wet-mash was prepared and weighed before the next rat was brought to the testing room. These procedures were followed for both the stress condition and the non-stress condition. Just like the verification tests, during the stress condition, the tone was turned on and maintained as soon as the lid of the chamber was closed. During the non-stress condition, the tone was absent.

Re-Training. Following experiment one, subjects were given four additional conditioning trials to make sure their learning had not extinguished during the one-choice testing procedures. The same procedures as described in training were used for this portion of the study.

Experiment 2: Two-Choice. Following experiment one, a second experiment was conducted where subjects could choose from two different wet-mashes at one time. This method is more popular amongst the literature for preference testing, as compared to one-choice tests. Since the subjects had a choice of what wet-mash they would prefer consuming, we had to account for the possibility that the subject may have chosen to completely avoid a wet-mash it disliked. This was why the first experiment was conducted.

Subjects were divided into two groups that were different than that of experiment one, but also consisted of an equal mix of CSE and CNE. The same procedures as experiment one were followed for experiment two, with the exception of how the wet-mashes were presented to the subjects. All 17 rats were exposed to a combination of two of three wet-mash mixtures at the same time: 0% and 10%, 0% and 60%, and 10% and 60%, while a high frequency tone was present (stress condition) and also while it was absent (non-stress condition). Before the

beginning of all sessions, 15g (10g of powder and 5ml of water) of each wet-mash (30g total wet-mash available to the subjects) was prepared and initial weights were recorded, so that wet-mash consumption could be accurately measured throughout the session. The bowls of wet-mash were weighed at three, six, and 10 minutes. At each time stamp, the location of the bowls was changed, to prevent the rats from associating a certain wet-mash flavour with a certain location within the chamber. Behavioural measures (rearing and grooming) were recorded throughout the trials. All trials lasted 10 minutes. The sessions in this experiment started in the exact same manner as experiment one and ended in the same way as well. Just as in experiment one, there was a stress condition where the tone was turned on as soon as the lid was secured and maintained for the duration of the session. As for the non-stress condition, the high frequency tone was muted throughout the duration of the session.

Results

An independent samples t-test was conducted to compare body weights between the two groups (conditioned stress non-eating: M = 326.78, SD = 15.97; conditioned stress-eating: M = 318.13, SD = 14.98) following random assignment, prior to the beginning of behavioural training. No significant differences were found between conditioned stress non-eaters and conditioned stress-eaters, $t(15) = -1.14$, $p = .83$. A second independent samples t-test was conducted to compare food intake between the two groups (conditioned stress non-eating: M = 27.88, SD = 5.62; conditioned stress-eating: M = 27.00, SD = 1.80) following random assignment, prior to the beginning of behavioural training. No significant differences were observed between conditioned stress-eaters and conditioned stress non-eaters, $t(15) = 0.44$, $p = .66$. Therefore, it was concluded that the two groups were statistically identical in body weight and food intake, prior to the beginning of their training.

Verification tests

A 2 (groups: conditioned stress non-eaters, conditioned stress-eaters) x 2 (conditions: stress, non-stress) x 3 (time intervals: 0-3min, 3-6min, 6-10min) mixed-design ANOVA was used to compare pellet consumption within each of the three time intervals, for conditioned stress non-eaters and conditioned stress-eaters, while the stressor was present and while it was absent. No significant main effects were found for group, $F(1,15) = .16$, $p = .69$, $\eta^2_p = .01$, for condition, $F(1,15) = .07$, $p = .79$, $\eta^2_p = .005$, or for time interval, $F(2,30) = .73$, $p = .49$, $\eta^2_p = .05$. However, a significant interaction of group, condition, and time interval was found (see Figure 1), $F(2,30) = 3.76$, $p < .05$, $\eta^2_p = .20$.

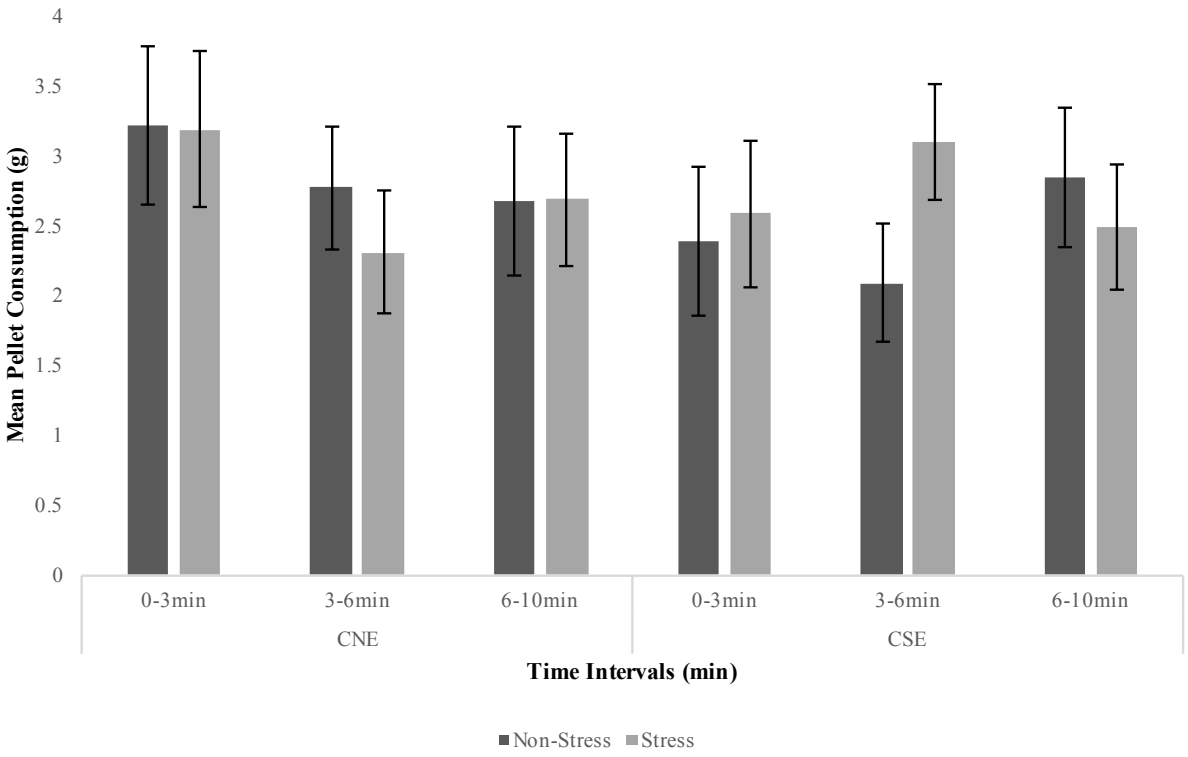


Figure 1. Mean pellet consumption (grams) over each time interval (min), for conditioned stress non-eaters and condition stress-eaters, during non-stress and stress conditions.

No other significant interactions were found. Contrasts were conducted to breakdown this interaction; however, no significant effects were revealed across all variables. This was likely due to a small sample size, resulting in a small observed power (.64).

A 2 (groups: conditioned stress non-eaters, conditioned stress-eaters) x 2 (conditions: stress, non-stress) mixed-design ANOVA was conducted to examine total pellet consumption at the end of 6-minutes and at the end of 10-minutes for the two groups, within the stress and non-stress conditions. For 6-minutes, results did not reveal any significant effects for group, $F(1,15) = .34$, $p = .57$, $\eta^2_p = .02$, or for condition, $F(1,15) = .66$, $p = .43$, $\eta^2_p = .042$. Although it was close, no significant interactions were found, $F(1,15) = 3.80$, $p = .07$, $\eta^2_p = .20$ (see Figure 2). For 10-minutes results did not reveal any significant effects for group, $F(1,15) = .16$, $p = .69$, $\eta^2_p = .01$, or for condition, $F(1,15) = .074$, $p = .79$, $\eta^2_p = .005$. In addition, no significant interactions were found between condition and group, $F(1,15) = .96$, $p = .34$, $\eta^2_p = .01$. Therefore, total pellet consumption at the end of 6-minutes and 10-minutes across conditions for both groups (see Figures 2 and 2.1).

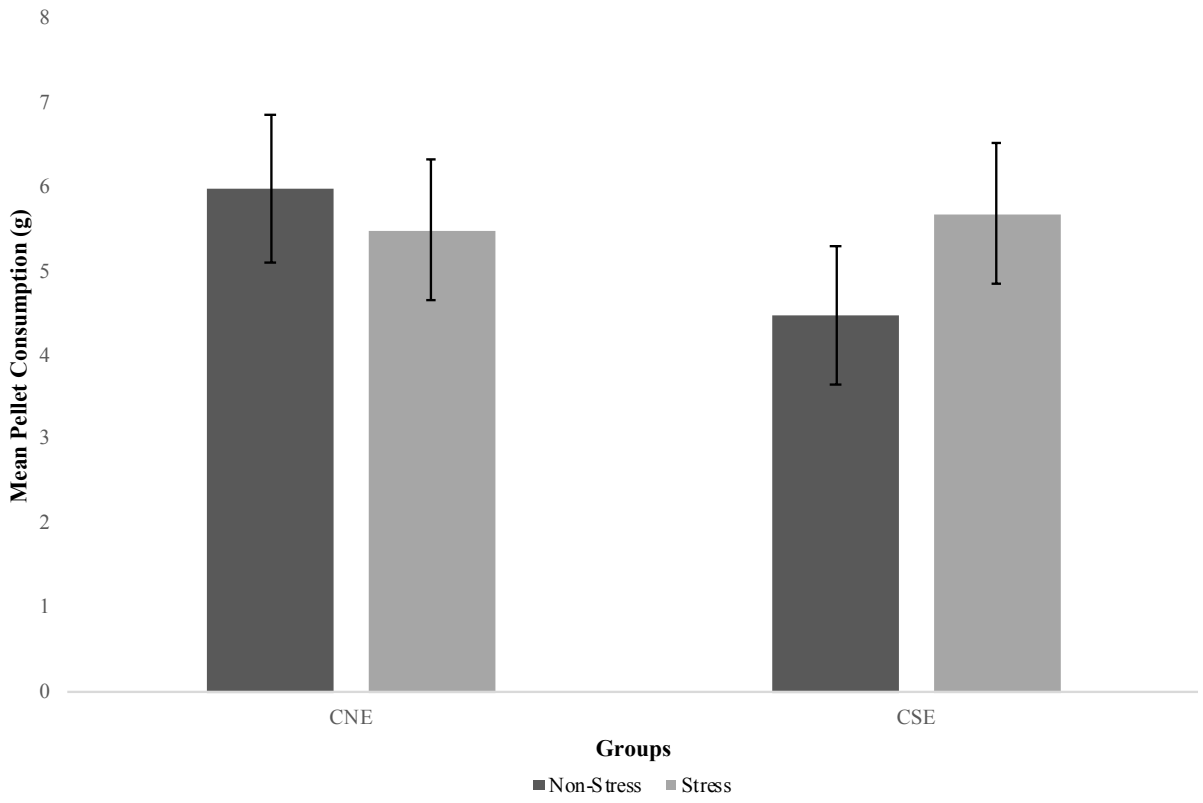


Figure 2. Total mean pellet consumption (grams) for CNE and CSE, in the stress and non-stress conditions, at the end of 6-minutes.

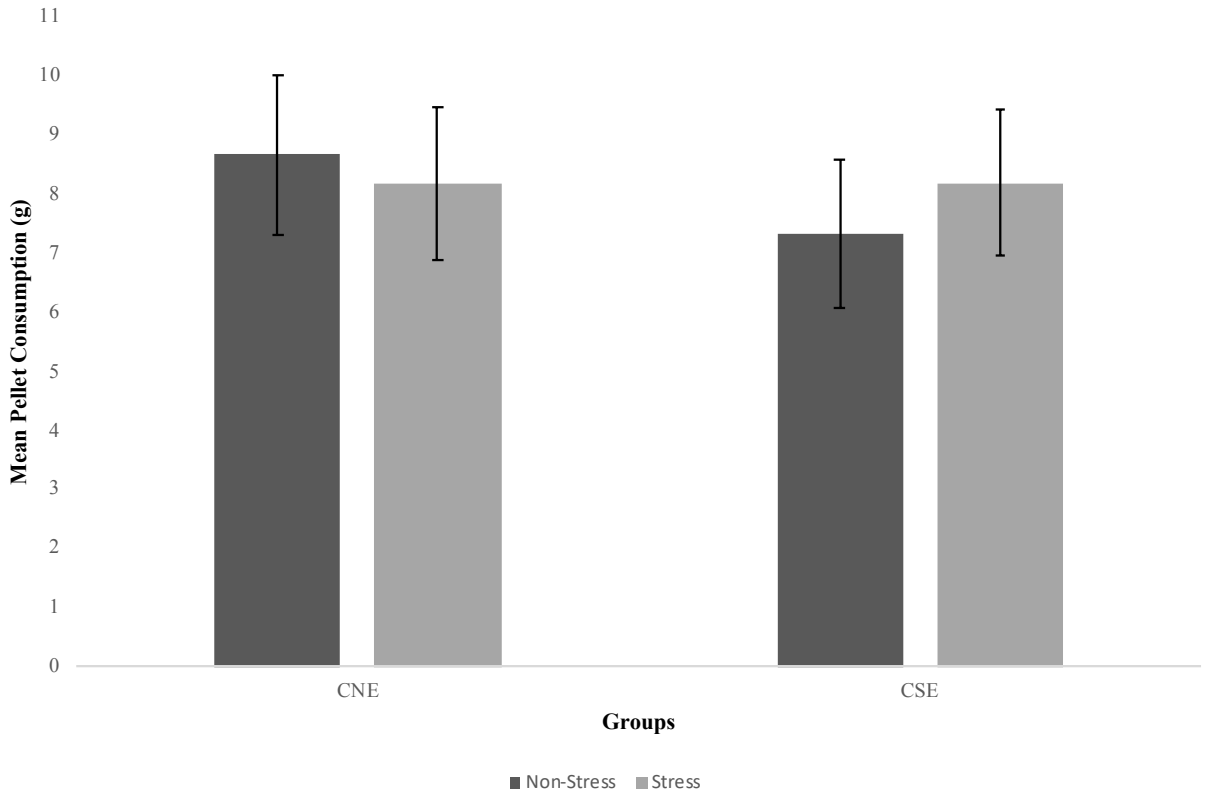


Figure 2.1. Total mean pellet consumption (grams) for CNE and CSE, in the non-stress and stress conditions at the end of 10-minutes.

Experiment 1: One-choice

A 2 (groups: conditioned stress non-eaters, conditioned stress-eaters) x 2 (conditions: stress, non-stress) x 3 (Splenda %: 0%, 10%, 60%) x 3 (time intervals: 0-3min, 3-6min, 6-10min) mixed-design ANOVA was computed to compare wet-mash intake between the 3 Splenda concentrations, within 3 time intervals, for conditioned stress non-eaters and conditioned stress-eaters, while the stressor was present and while it was absent. Results revealed a significant main effects of Splenda %, $F(2,30) = 177.88$, $p < .01$, $\eta^2_p = .92$, and of time interval, $F(2,30) = 21.99$, $p < .001$, $\eta^2_p = .55$. There were no significant main effects found for group, $F(1,15) = .23$, $p = .64$, $\eta^2_p = .015$, or for condition, $F(1,15) = .28$, $p = .61$, $\eta^2_p = .018$.

A significant interaction was found between Splenda % and time interval (see Figure 3), $F(4,60) = 18.61$, $p < .01$, $\eta^2_p = .55$.

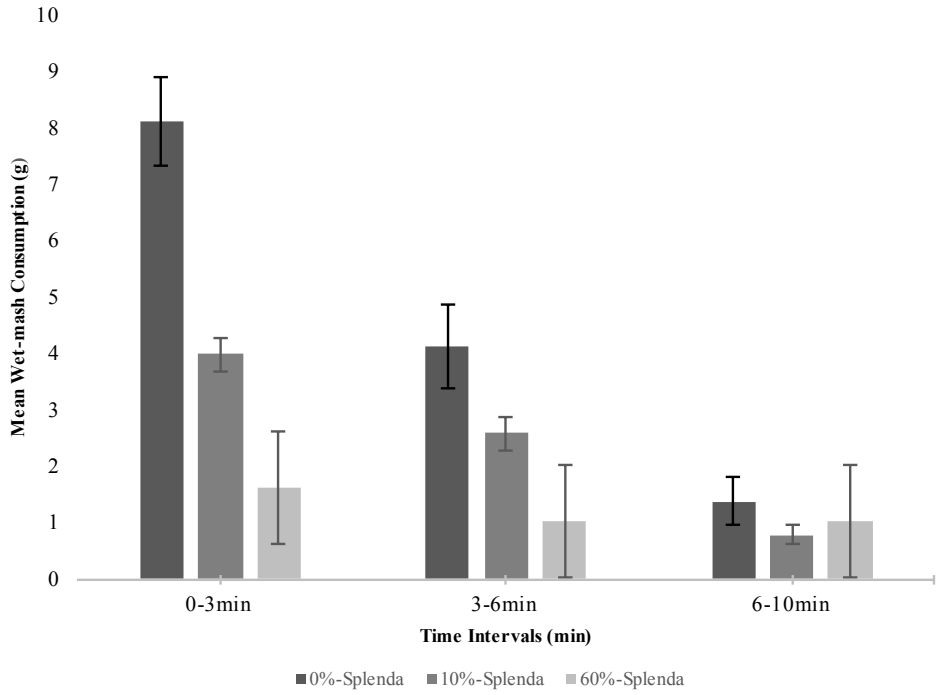


Figure 3. Mean wet-mash consumption (grams) over time (min) for both group and conditions combined, for each Splenda %.

Contrasts were explored and it was found that for both 0%-Splenda and 10%-Splenda, more wet-mash was consumed within the 0-3min interval (M = 8.12, SD = .78; M = 4.12, SD = .74), as compared to both the 3-6min interval (M = 3.98, SD = .29; M = 2.58, SD = .29) and the 6-10min interval (M = 1.62, SD = .37; M = 1.01, SD = .14). Additionally, for both the 0%-Splenda and 10%-Splenda wet-mashes, significantly more wet-mash was consumed within the 3-6min interval (M = 3.98, SD = .29; M = 2.58, SD = .29) as compared to the 6-10min interval (M = 1.62, SD = .37; M = 1.01, SD = .14). There were no significant differences found between time intervals for the 60%-Splenda wet-mash, indicating that mash consumption was similar across all time intervals. No additional significant interactions were found.

A 2 (groups: conditioned stress non-eating, conditioned stress-eating) x 2 (conditions: stress, non-stress), x 3 (Splenda %: 0%, 10%, 60%) was conducted to examine total mash consumption between the 3 Splenda concentrations, for the two groups, while the stressor was present, and while it was absent. There was a main effect of Splenda %, $F(2,30) = 177.88$, $p < .001$, $\eta^2_p = .92$, but no significant main effects were found for condition, $F(1,15) = .28$, $p = .61$, $\eta^2_p = .018$, or for group, $F(1,15) = .23$, $p = .63$, $\eta^2_p = .015$ (see Figure 4).

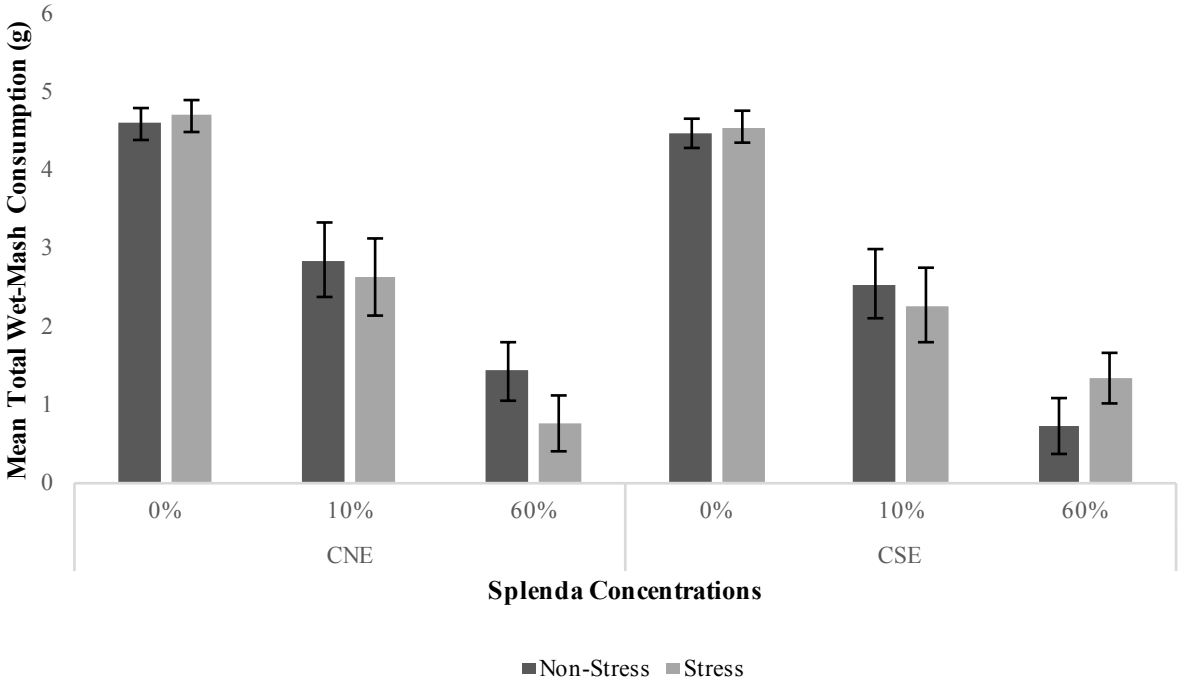


Figure 4. Total mean wet-mash consumption (grams) for CNE and CSE, between both conditions, for each Splenda-%, after 10-minutes.

Additionally, there were no significant interactions found. For the significant main effect of Splenda %, contrasts revealed that subjects consumed significantly more 0%-Splenda wet-mash (M = 12.72, SD = .35) than both 10% (M = 7.71, SD = .85) and 60%-Splenda wet-mashes (M =3.19, SD = .61). Furthermore, subjects also consumed significantly more 10%-Splenda wet-mash (M = 7.71, SD = .85) than 60%-Splenda wet-mash (M =3.19, SD = .61). This indicates that overall, all subjects consumed more wet-mash when it contains less Splenda.

An exploratory 2 (groups: conditioned stress non-eaters, conditioned stress-eaters) x 2 (conditions: stress, non-stress) x 2 (behaviours: rearing, grooming) x 3 (Splenda %: 0%, 10%, 60%) mixed-design ANOVA was conducted to look at the effects of stress on stress-related behaviours. Results revealed a significant main effect for condition (see Figure 5), $F(1,15) = 4.82$, $p < .05$, $\eta^2_p = .24$.

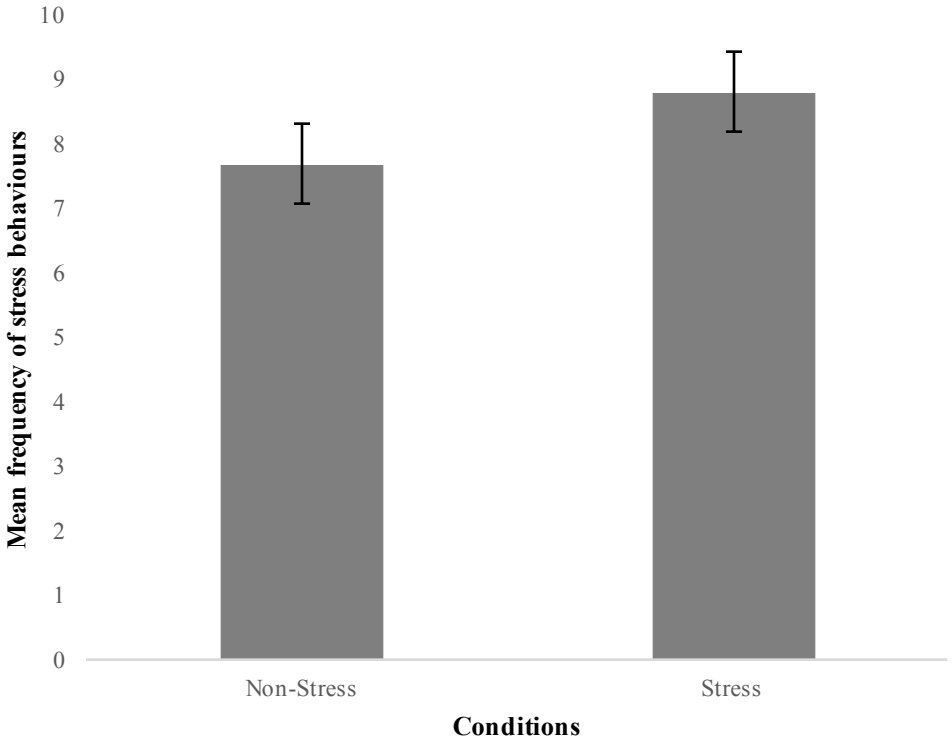


Figure 5. Mean frequencies of stress-related behaviours (rearing and grooming combined), in the stress and non-stress conditions, for both groups.

Subjects from both groups executed higher frequencies of both rearing and grooming combined, during the stress condition (M = 8.81, SD = .62) as compared to the non-stress condition (M = 7.69, SD = .62). This indicates that the stressor (tone) did affect other behaviours that were not feeding. Significant main effects of behaviour, $F(1,15) = 40.32$, $p < .001$, η^2_p = .73, and Splenda %, $F(2,30) = 33.45$, $p < .001$, $\eta^2_p = .69$, were also found. More rearing and grooming was observed with wet-mashes that contained more Splenda. As shown previously, subjects consumed less wet-mash the more Splenda it contained. Therefore, less feeding resulted in more time spent performing other behaviours.

Experiment 2: Two-Choice

For the data obtained from the two-choice experiment, total consumption of each individual wet-mash was analyzed, within the three wet-mash combinations. For the purpose of this analysis and relating figures, wet-mashes were labeled as: combo1 (0% and 10%), combo2 (0% and 60%), and combo3 (10% and 60%). A 2 (groups: conditioned stress non-eaters, conditioned stress-eaters) x 2 (conditions: stress, non-stress) x 6 (wet-mash: combo1[0%, 10%], combo2 [0%, 10%], combo3 [10%, 60%]) mixed-design ANOVA was conducted to compare total wet-mash consumption between each of the individual mixtures within a combination, for CNE and CSE, while a stressor was present and also when it was absent.

Results did not reveal any significant main effects for condition, $F(1,15) = 2.43$, $p = .14$, $\eta^2_p = .14$, or for group, $F(1,15) = .003$, $p = .96$, $\eta^2_p = .00$. However, there was a significant interaction found between condition and group (see Figure 6), $F(1,15) = 7.18$, $p < .05$, $\eta^2_p = .32$.

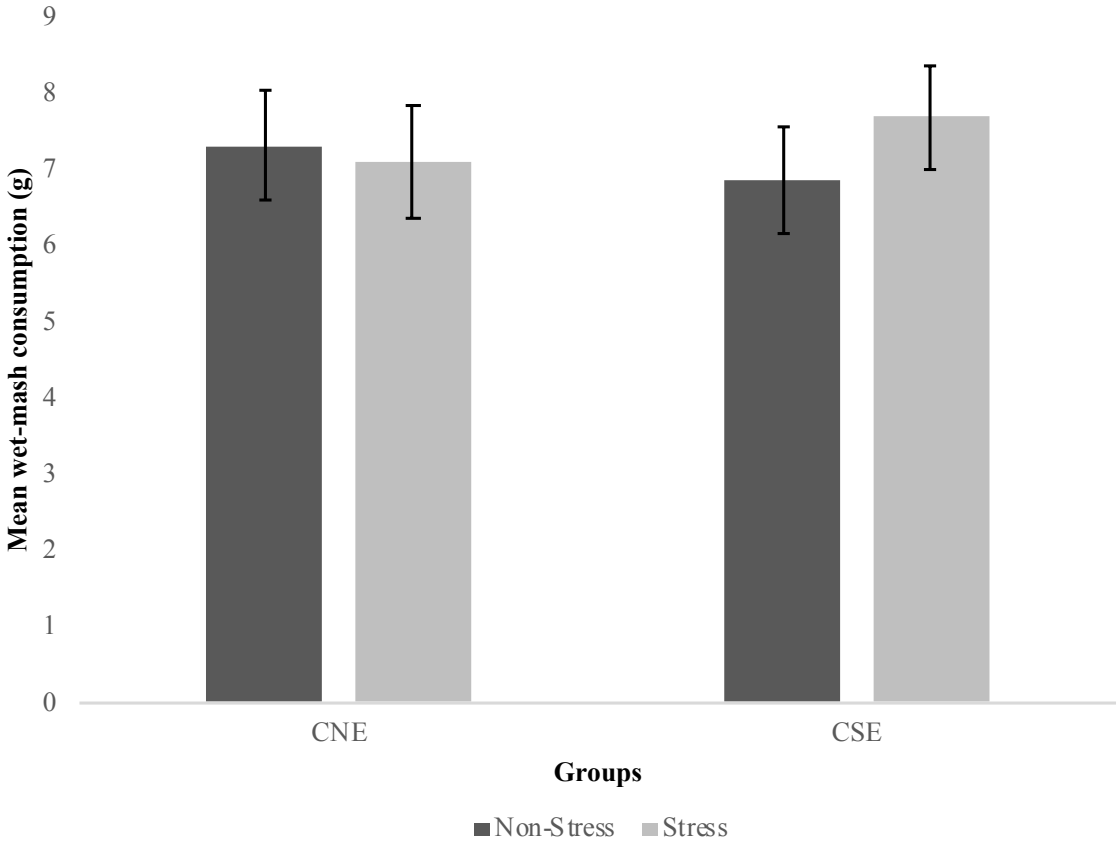


Figure 6. Mean wet-mash consumption (grams) for CNE and CSE in the stress and non-stress conditions.

Conditioned stress-eaters consumed more overall wet-mash in the stress condition (M = 7.68, SD = .70), as compared to the non-stress condition (M = 6.85, SD = .69). On the other hand, conditioned stress non-eaters' overall chow consumption remained similar across both the stress (M = 7.10, SD = .74) and non-stress (M = 7.32, SD = .74) conditions. This indicates that conditioned stress-eaters increased their food consumption while stressed, as compared to non-stressed, which is in line with one of the study's predictions. However, conditioned stress non-eaters kept their feeding the same in both conditions.

Additionally, results showed that there was a significant main effect of wet-mash type (see Figure 7), $F(5,75) = 117.92$, $p < .001$, $\eta^2_p = .89$.

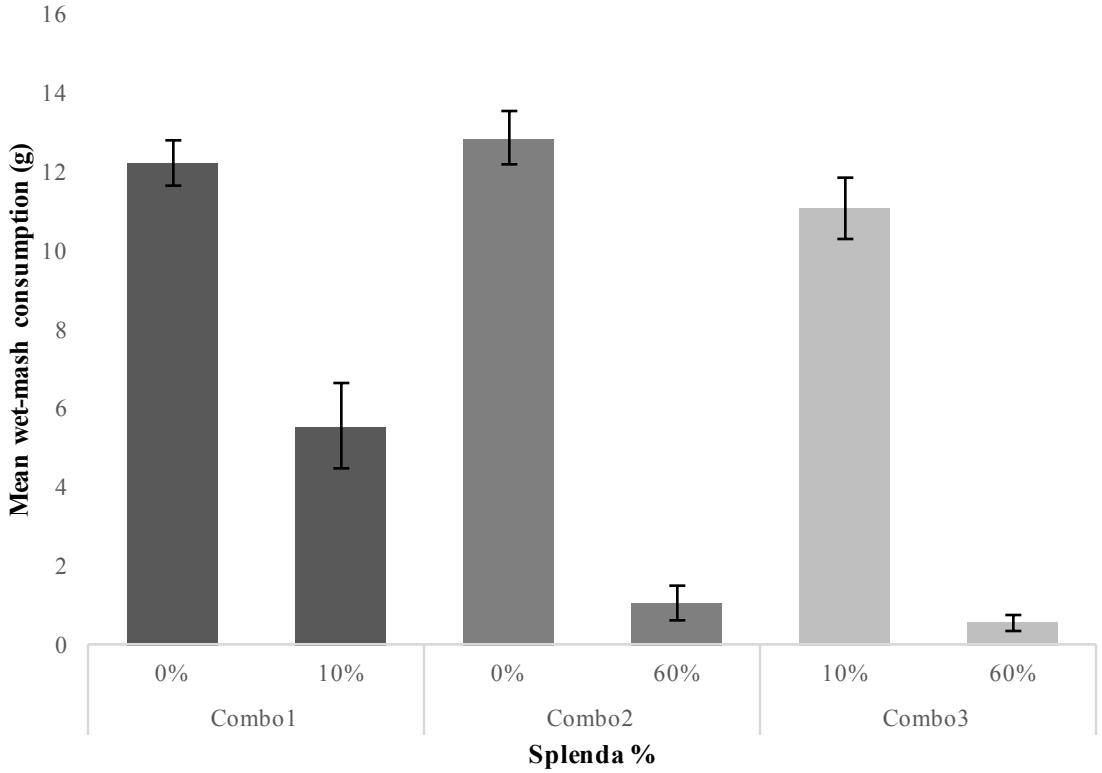


Figure 7. Mean individual total chow consumption (grams) within all combinations for both groups, in the stress and non-stress conditions.

Starting with combo1 (0%-10%), contrasts revealed that significantly more 0%-Splenda wet-mash was consumed (M =12.23, SD = .55) than 10%-Splenda wet-mash (M = 5.57, SD = 1.10). For the combo2 (0%-60%), contrasts revealed that once again, significantly more 0%-Splenda wet-mash was consumed (M = 12.85, SD = .67) than 60%-Splenda wet-mash (M = 1.90, SD = .45). Finally, for combo3 (10%-60%), contrasts revealed that significantly more 10%-Splenda wet-mash was consumed (M = 11.09, SD = .79), as compared to 60%-Splenda wet-mash (M = .59, SD = .22). This indicates that within all three combinations, rats from both groups preferred the mixture with the least amount of Splenda added (0%), as shown by their increase in intake, as compared to the other mixture present. When the 0%-Splenda wet-mash was not available, rats turned to the 10%-Splenda wet-mash.

Finally, since two different bowls of chow were being measured at the same time during this experiment, it was of interest to examine delta values (differences). These were calculated by subtracting the total wet-mash consumption of one bowl, from the other. These values were then used to conduct the following analysis.

A 2 (groups: conditioned stress non-eaters, conditioned stress-eaters) x 2 (conditions: stress, non-stress) x 3 (mash combination: 0%-10%, 0%-60%, 10%-60%) mixed-design ANOVA was conducted to examine wet-mash consumption differences, for each group and across conditions. Results revealed a significant main effect of wet-mash combination (see Figure 8), $F(2,30) = 12.62$, $p < .001$, $\eta^2_p = .46$.

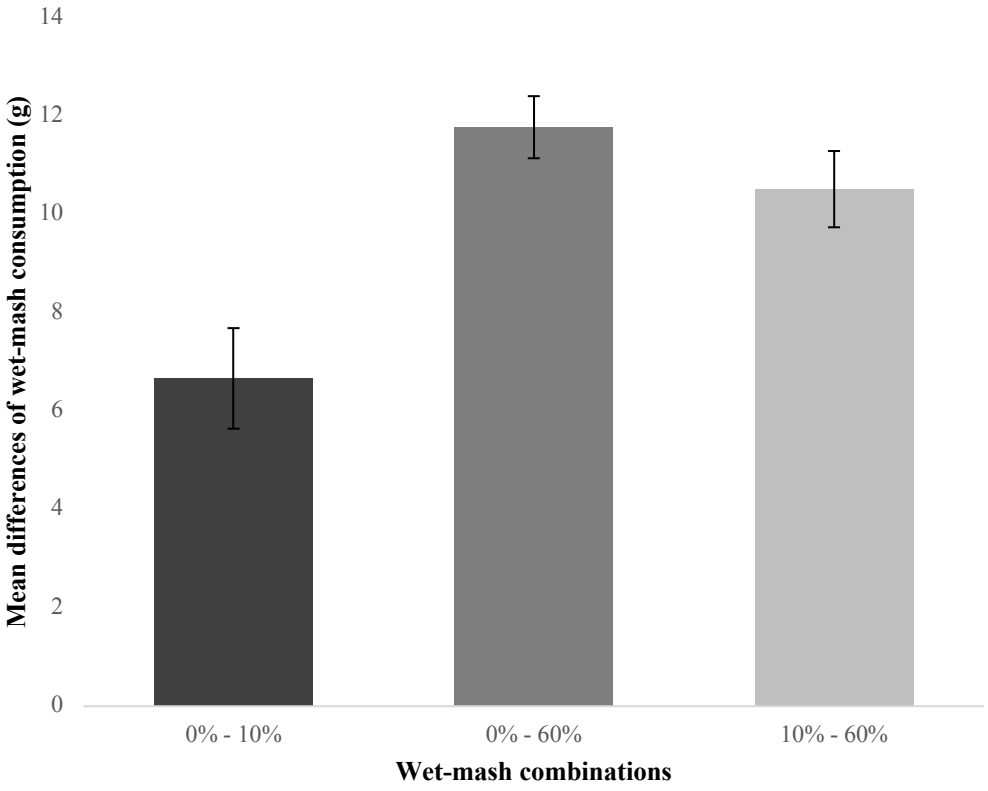


Figure 8. Mean differences of wet-mash consumption (grams) between each of the three wet-mash combinations.

Contrasts were conducted, and it was found that the wet-mash intake differences in the 0%-60%-Splenda combination (M = 11.76, SD = .63) were significantly higher than both the 0%-10%-Splenda combination (M = 6.66, SD = 1.02) and the 10%-60%-Splenda combination (M = 10.51, SD = .76). Additionally, significant wet-mash intake differences were observed between the 10%-60%-Splenda combination (M = 10.51, SD = .76) and the 0%-10%-Splenda combination (M = 6.66, SD = 1.02), whereby significantly larger differences in wet-mash intake were observed within the 10%-60%-Splenda combination, as compared to the 0%-10%-Splenda combination. There was no other significant main effects found for both condition, $F(1,15) = .29$, $p = .59$, $\eta^2_p = .019$, and group, $F(1,15) = 1.22$, $p = .29$, $\eta^2_p = .075$. Thus, wet-mash preferences for both groups did not change whether the subjects were stressed or not. Furthermore, no significant interactions were revealed.

Discussion

The stress response recruits many different mechanisms within the body and brain, which work together in an attempt to help the organism recover from a stressful situation and return it to homeostasis. As such, both behavioural and psychological changes occur within an organism. One of these changes, which was most relevant to the current study, is feeding behaviour. It is now clear that, in both humans and animals, the feeding response is affected in a bidirectional manner by stress. This means that some increase, while others decrease their food intake in response to stress. Research has attempted to find specific factors contributing to this divergence, only to yield mixed results (Greeno & Wing, 1994).

In addition to different learning histories, palatability of food was also an important factor to consider within the scope of stress-induced feeding changes. As mentioned previously, it has been observed in both humans and animals, that when experiencing stress, both seem to increase their intake of the more palatable foods (ex. sweeter or saltier) as compared to blander foods. It is believed that this is because the ingestion of palatable foods interacts with the brain's reward system, by releasing endogenous opioids, which then mitigates the negative affect of the stress response (Drolet, et. al., 2001; Kreek & Koob, 1998; O'Hare, et. al., 2004; Pecoraro et al., 2004). Nonetheless, it is still unclear if or how stress-eaters and stress non-eaters differ in their food preference while experiencing stress.

Current Study

The purpose of the current study was to demonstrate that the stress-induced bidirectional feeding response observed in humans, could be due to different learnt associations between a stressor and the act of eating food. By using operant conditioning, the goal was to create an animal model of stress-eating and stress non-eating as suggested by Johnson and Emond (2017).

Johnson and Emond (2017) demonstrated that rats could effectively be trained to increase or decrease their food intake in the presence of various stressors including: foot shock, restraint, tail pinch, and a high-frequency tone. The current study employed the use of a high-frequency tone as its aversive stimulus and stressor. High-frequency tones have been shown in research to reliably produce a stress response and other behavioural changes in lab animals (Chandralekha, Jeganathan, Viswanathan, & Charan, 2005; Johnson & Emond, 2017; Krebs, Macht, Weyers, Weijers, & Janke, 1996; Monjan & Collector, 1977; Weingarten, 1985). In addition, noise stressors have been shown to produce changes in the eating behaviour of lab animals, which was particularly relevant to the current study (Krebs et al., 1996).

Negative reinforcement was used to train nine of the seventeen rats to associate feeding with the removal of a negative outcome. The goal was to increase the probability for a behaviour (feeding) to occur. This experimental group was labelled the Conditioned Stress-Eaters (CSE). Punishment training was used to train the remaining eight rats to associate feeding with the presentation of a stressor. The goal was to decrease the probability for a behaviour (feeding) to occur. This experimental group was labelled the Conditioned Stress Non-Eaters (CNE). Although there was a similar goal of demonstrating that stress-eating and stress non-eating may be a result of different past learning histories, it is important to note that the current study changed a couple of key components from the methodology used by Johnson and Emond (2017). These changes included: the rat strain (Wistar vs. Sprague-Dawley), and the reward pellets (Sucrose vs. un-sweetened). Rat strain was altered because in addition to being a popular animal strain used in research, it was of interest to explore if this model could be generalized to other strains of rats. Furthermore, unsweetened pellets were used for training so that the animals did

not habituate to any "sweet taste" and for this flavour to remain novel to the animals for the palatability portion of the study.

The second portion of this study was aimed at applying the above animal model of stress-eating and non-eating, and examine their feeding responses in the presence of various levels of sweetened chows while a stressor was present and while it was absent absent. It was also of interest to examine if palatable food preferences would change for these groups, in the presence and absence of a stressor. Three different "wet-mashes" were created by mixing powdered training pellets and adding different amounts of Splenda. These Splenda concentrations were 0%, 10%, and 60%. As mentioned previously, Splenda is a sucralose-based sweetener that was chosen for this study because of its non-caloric properties and its widespread use in human foods and beverages. This allowed for direct changes in palatability (increases in sweetness), without increasing the calorie content of the wet-mashes. The main reason for choosing a non-caloric sweetener to alter the mashes' palatability, was to limit post-ingestion effects of calories. It has been shown in research that calories provided by food, have rewarding properties, and can therefore reinforce feeding behaviour (Volkow, Wang, & Baler, 2011). Thus, for the purpose of this study, it was important to isolate the hedonic component of food (palatability), from their energy content (calories).

The subjects were exposed to the three chows in two different ways. The first was by means of the one-choice test, where each animal was presented with one of the three chow mixtures at a time. Chow intake was measured, while the stressor was present, and while it was absent. The second, was by means of a two-choice test. Rats were presented with combinations of 2/3 chow formulas: 1) 0%-10%, 2) 0%-60%, and 3) 10%-60%. Similar to the one-choice test, chow consumption was recorded when the stressor was present, and when it was absent. The

two-choice test allowed for direct measurement of chow preference, since rats were able to choose which chow it wanted to consume, and/or avoid. On the other hand, the one-choice test allowed for measurements of chow intake, without influences from other chow flavours.

It was anticipated that the results of this study would help expand the current understandings of how changes in stress and food palatability affect the eating response in rats and provide more clarification as to what factors contribute to the bidirectional, stress-induced feeding responses observed in humans. The current study sought to demonstrate through an animal model, that these two distinct populations are a result of different learning histories. Additionally, the current study sought to add to the current literature on palatability and stress, by exploring food preferences of the animal model described above.

First, it is important to note that once the animals were assigned to their experimental group, statistical analyses compared their average weights and food intake. It was confirmed that both groups were statistically equal prior to the start of the experiments.

Verification Tests

The verification tests were conducted following the end of behavioural training sessions, in order to verify that training had successfully shaped two distinct groups: one that responds to stress by increasing their food intake (CSE), and another that decreases their food intake in response to stress (CNE). With this in mind, it was hypothesized that the CSE group would increase their food intake, while the CNE would decrease their food intake in the presence of a high-frequency tone (stressor). On the other hand, when the stressor was absent, their feeding behaviour would be at baseline. These predictions were based on a previous study conducted by Johnson and Emond (2017), which demonstrated that stress-induced eating and non-eating could

successfully be shaped using operant conditioning (negative reinforcement and punishment training), and a high-frequency tone as the aversive stimulus.

Although it was not statistically significant, the results of the verification tests revealed specific differences in feeding behaviour between CNE and CSE as a response to the stressor, within one of the time intervals. Referring back to Figure 1, it was found that CSEs increased their feeding when the stressor was present as compared to when it was absent, during the 3-6min interval. This tendency was partially in line with the hypotheses stated previously. This was not the case for the CNEs, who kept their feeding behaviour relatively similar in both the stress and non-stress conditions. Statistical significance might have been observed for this group, had the sample size been bigger. Moreover, cumulative food intake between both groups, in both conditions, was examined at the end of 6-minutes and 10-minutes. No differences in food intake were found between groups, conditions, or time stamp. Despite the absence of statistical significance (although it was close), at the end of 6-minutes, the desired feeding behaviour pattern for CSEs in response to stress was observed. Figure 2, shows that in the stress condition, CSEs increase their cumulative food intake as compared to the non-stress condition. Once again, an increase in sample size might have been sufficient in yielding significant results.

The training procedures for this study were based on the methodology of Johnson and Emond (2017). However, the present study changed a few components that might have contributed to results observed in the verification tests. These key changes included: rat strain, training pellets, and sample size. While Johnson and Emond (2017) used male Wistar rats, the present study used male Sprague-Dawley rats. Sprague-Dawley rats are a well-known strain for animal models in research, because of their calm temperament. Due to this trait, it is possible that this strain was less sensitive to the stressor as compared to Wistar rats. Even though high-

frequency tones have shown to be reliable stressors for lab animals (Johnson & Emond, 2017; Monjan & Collector, 1977; Weingarten, 1985), it seems as though a more intense stressor might have been needed elicit a stress response strong enough to affect feeding behaviour, in this particular strain of rats.

On another note, since the animals were food deprived prior to the tests, it is possible that their instinctual drive to replenish their energy needs (homeostatic hunger), was competing with their physiological stress response. In other words, the rats might have been more physiologically concerned with satiating themselves than responding to the stressor. Additionally, anorectic effects of the typical stress (fight or flight) response were not observed in this experiment, perhaps because the stress response was milder than hunger, as demonstrated by similar food intake amounts when the stressor was absent.

Moreover, while it is more common to use sweetened precision-pellets as reward in learning paradigms, the precision-pellets in this study were bland in flavour (unsweetened). This was done because food palatability was being explored later in the study, by means of adding Splenda (a sweetener) to the food. The goal was to eliminate possible habituation to sweet taste, brought upon by sucrose precision-pellets. Although the rats seem to like the taste precision-pellets used for training (as demonstrated by the fact they were consuming what was given to them), it is possible that they were not as rewarding as the typical sucrose precision-pellets.

In sum, the training procedures employed in the current study, yielded slight differences in food intake for CSEs when the stressor was present as compared to when it was absent. This was not the case for CNE's who kept their food intake similar regardless of condition. Nonetheless, the study continued into Experiment 1, to examine if more obvious stress-induced differences in food intake, for the two groups would emerge in the presence of palatable food.

Experiment 1: One-Choice

The one-choice test was conducted to examine chow consumption for each of the three Splenda concentrations (0%, 10%, and 60%) for CNE and CSE, in the presence and absence of a stressor. The different chows were presented to the subjects individually, in order to measure consumption without the possible influences of other the other chow mixtures. Similar to the verification tests, it was first hypothesized that when the stressor was present, CSEs would increase their overall food intake, while CNEs would decrease their overall food intake, as compared to when the stressor was absent. Moreover, when the stressor was absent, feeding for both groups would be at baseline.

Secondly, it was hypothesized that while the stressor was absent, both groups would show a preference for mixtures containing more Splenda (10% and 60%) as depicted by an increase in intake, compared to the 0%-Splenda mix. As discussed previously, this hypothesis was based on the fact that in normal circumstances, sweet solutions are generally enjoyed by lab animals as compared to other flavours (Berridge, 1996; Levi et. al., 2014; Pecoraro et. al., 2004; Young, 1966). Furthermore, under normal circumstances, highly palatable foods (such as those containing sugar) have been shown to trigger an appetizing effect, where appetite is increased as food is eaten, therefore increasing food intake (Le Magnen, 1985; Yeomans, 1998). More specifically, when the stressor was absent, it was hypothesized that both groups would prefer the 10%-Splenda chow. This prediction was based on a study by Levi et. al. (2014), which demonstrated that in normal conditions, rats responded more to a solution sweetened at 25% --- the middle concentration, compared to 8% and 50%.

Thirdly, it was hypothesized that when the stressor was present, there would be a noticeable preference for 60%-Splenda chow for CSEs. This stress-induced shift in preference

was expected since it has been shown in research that stress can decrease the rewarding properties of palatable foods, therefore, palatability would need to be increased in order to produce the same pleasurable effects (Born et. al., 2010). Additionally, since CSEs should have learned that eating results in the removal of a stressor, the comforting effects of eating should be amplified with 60%-Splenda chow.

The first set of analyses from this experiment compared food consumption, when the stressor was present, and when it was absent, for each of the three Splenda concentrations, over time, for CNEs and CSEs. Results revealed that, regardless of group assignment or condition (stress or non-stress), the highest quantities of chow were consumed in the first 3 minutes of the sessions for both 0%-Splenda and 10%-Splenda. However, this was not observed for 60%-Splenda mixture, where consumption amounts remained relatively similar throughout every time interval. Since the rats were food restricted for 12hrs prior to testing, and should have been hungry at the beginning of the sessions, it made sense that the most chow was eaten within the first 3 minutes. However, this pattern should have been observed across all Splenda %, to some extent. A possible explanation for this absence of "bingeing" effect with the 60%-Splenda chow, could be that rats found this particular concentration very undesirable, and thus spent their time avoiding the chow. As seen in Figure 4, it is clear that consumption amounts for 60%-Splenda were drastically lower than that of the other two concentrations. Another analysis was conducted to explore cumulative chow consumption for each of the three Splenda mixes at the end of 10-minutes (end of session).

When looking at total chow consumption (i.e. at the end of 10min) for CNEs and CSEs, between each condition, it was found that once again all subjects consumed more 0%-Splenda chow than both the 10%-Splenda and 60%-Splenda chows, regardless of condition. This

suggested that they preferred a chow containing no Splenda at all, as compared to the two chows containing Splenda. As a reminder, these Splenda concentrations were chosen to ensure that there were sufficient differences in taste to be detected by the animals. These results contradicted what was found in some literature, where it was suggested sweet taste is generally liked by lab animals (Berridge, 1996; Levi, et. al., 2014; Young, 1966) and that increasing the concentration of sweetener in food should have increased its palatability, resulting in an increase in food intake (Yeomans, 1998; Young, 1966). Interestingly, the results of this experiment displayed a similar pattern as what was found by Yeomans (1998) with salt; this flavour can increase or decrease palatability depending on its concentration in food. Much like saltiness, sweet taste can also have a bidirectional effect on palatability depending on its concentration (Monneuse, et. al.,1991; Perez, Dalix, Guy-Grand, & Bellisle, 1994). Therefore, it is quite possible that the rats thought that the chosen concentrations of Splenda were too sweet, which made the chows undesirable, resulting in a decrease in intake. This phenomenon has been observed in human research, where higher concentrations of sweetener in food, resulted in reduced hedonic ratings from subjects. A study by Monneuse et al. (1991) looked at men and women's hedonic ratings of plain yogurt containing five different concentrations of aspartame. Much like sucralose, which is the main ingredient in Splenda, aspartame is an intense, non-caloric sweetener, which is often used in food and beverages as an alternative to sugar. They found that hedonic ratings were highest for yogurts containing the lower concentrations of aspartame, and the lowest ratings were given to yogurt containing the highest concentrations. Furthermore, food intake was lower for those higher concentrations (rated as less palatable), and higher for the lower concentrations (rated as more palatable) (Monneuse et al., 1991). A similar study was conducted by Perez et al. (1994), where men and women had to rate the taste of plain yogurt containing five different

concentrations sucrose (table sugar). Results revealed that hedonic ratings increased as sucrose concentrations increased, but after the 3rd concentration (10%), hedonic ratings decreased as concentrations of sucrose increased. They also measured yogurt intake for each of the five concentrations and found that intake was the greatest for 10%-sucrose (preferred concentration) in men only. Both of these studies were conducted under normal (non-stress) conditions.

The aversion to both Splenda mixtures exhibited by all subjects, which was not observed for the mix containing 0% Splenda, could also mean that the rats disliked the taste of the sweetener itself. This idea was investigated in the literature and it was suggested that most rats are actually sensitive to an off-taste found in sucralose (main ingredient in Splenda), which would significantly decrease its palatability (Loney, Torregrossa, Smith, Scalfani, and Eckel, 2011; Scalfani et, al., 2004). This idea will be discussed in more detail within the limitations section.

Furthermore, despite the addition of new flavours of chow, results of the one-choice test were similar to that of the verification tests, in that feeding behaviour for CNEs and CSEs was unaffected by the presence of a stressor. Possible reasons as to why this occurred were explained in detail within the verification tests section, whereby homeostatic hunger (replenishing the body's energy needs) might have taken precedence over the physiological effects of stress. On the other hand, the specific strain of rats (which have a calm temperament) could have been insensitive to the effects of the chosen stressor for this study. Furthermore, there was no stress-induced preference for chows containing higher concentrations of Splenda in either of the groups. All subjects preferred 0%-Splenda chow over the other two concentrations when the stressor was present and when it was absent. These results contradict some past literature, where it was suggested that stress can produce an increase in palatable food consumption due to its

positive effects on the stress response (Adam & Epel, 2007; Drolet, et. al., 2001; Kreek & Koob, 1998; O'Hare, et. al., 2004; Pecoraro et al., 2004). However, they were in line with what was previously mentioned about the fact that the chosen Splenda concentrations, were likely too sweet and thus became undesirable, and resulted in a decrease in intake regardless (Monneuse, et. al., 1991; Perez, et. al., 1994).

The last set of analyses for this experiment were exploratory, and not part of the original hypotheses. Behaviours other than feeding (rearing and grooming) were compared between groups and conditions, to see if the high-frequency tone was producing a stress response in these animals. It was confirmed that both rearing and grooming combined, increased in the presence of the stressor (as compared to when it was absent) for all subjects. Thus, the stressor seemed to be effective at altering some behaviours other than feeding (Krebs, et. al., 1996). Additionally, the fact that there were significant changes in behaviour in response to the stressor at this point in the study, suggested that the subjects were not habituating to the stressor.

Experiment 2: Two-Choice

The second experiment involved a two-choice test, where subjects were presented with two of the three Splenda concentrations at the same time. With this test, subjects were able to make a choice as to which Splenda concentration they preferred to eat. This allowed for observation of chow preferences (via food intake) for CNEs and CSEs, when a stressor was present and when it was absent.

The hypotheses for this experiment were similar to that of Experiment 1. First, it was hypothesized that when the stressor was present, CSEs would increase their combined overall chow intake (0%, 10%, and 60%), while CNEs would decrease their combined overall chow

intake (0%, 10%, and 60%), as compared to when the stressor was absent. When the stressor was absent, it was hypothesized that both groups would exhibit their baseline feeding behaviour.

In regard to their chow preferences, it was predicted that there would be a stress-induced preference for chow containing the highest concentrations of Splenda between the two choices. This means that in combination 1 (0%-10%), subjects were expected to prefer 10%-Splenda over 0%-Splenda, in combination 2 (0%-60%), they would prefer 60%-Splenda over 0%-Splenda, and in combination 3 (10%-60%), they would prefer 60%-Splenda over 10%-Splenda mix. The largest difference in mash intake within a combination should be an indication of the preferred chow and the least preferred chow. Moreover, it was predicted that CSEs would show a notable stress-induced preference for chow containing the most Splenda within each combination. As explained previously in Experiment 1, this was hypothesized because this group should have learned to associate feeding with the removal of a stressor and should show increased preference for a sweeter chow because stress has been shown to decrease the rewarding properties of palatable food. Thus, in theory, a sweeter chow should be required to provide the same positive effects of ingesting palatable chow during stress, as compared to a non-stressed condition.

Finally, when the stressor was absent, it was predicted that there would be a unanimous preference for the 10%-Splenda chow (or 0%-Splenda in combination 2). Reasons behind this prediction were discussed in detail in experiment 1, but to reiterate, under normal circumstances, rats have been shown to prefer a mid-sweet concentration over one that is too high or too low.

Results revealed that CSEs consumed more overall chow (0%, 10%, 60%) when the stressor was present, as compared to when the stressor was absent. However, this was not observed with the CNEs, who kept their overall chow intake similar across the stress and non-stress conditions. These results were partially in line with what was hypothesized from the very

beginning, where it was predicted that stress would elicit an increase in feeding for CSEs, due to the fact that with prior training, they would have learned to associate feeding with the removal of a stressor. This is what was demonstrated (in part) within the Johnson and Emond (2017) study. Additionally, these findings were consistent with what has been found in literature, where stress can change feeding behaviour (Adam & Epel, 2007; Antelman & Szechtman, 1975; Calvez et al., 2011; Dallman, 2010; Hawkins et al., 1992; Levine & Morley, 1981; Marti et al., 1994; Pecoraro et al., 2004). These findings also suggest that CSEs show more vulnerability to changes in feeding behaviour in response to stress, as compared to CNEs, who did not change their food intake as a result of stress. Due to their previous training experiences, feeding in response to stress likely provided some relief from the stress brought upon by the high-frequency tone. In addition to this stress relief, feeding resulted in the removal of hunger (animals were food deprived).

On the other hand, CNEs had a different experience in training, whereby eating resulted in the presentation of the aversive stimulus. It is possible their overall consumption did not change because for one, they learned that eating would not make the stressor go away, and two, homeostatic hunger might have overridden the drive to remove the stressor. As explained earlier, intense hunger could have been more distressing to the animals, than the stress response triggered by the high-frequency tone. Thus, the anorectic effects typically brought upon by the stress response (Calvez et al., 2011; Marti et al., 1994), were not observed in this particular case.

Proceeding to chow preferences between groups, similar results as Experiment 1 were observed. Regardless of whether or not the stressor was present, rats consistently preferred chows containing the lowest concentrations of Splenda within a combination. Thus, in combination 1 (0%-10%), consumption of 0%-Splenda wet-mash was much higher than that of

10%-Splenda wet-mash. In combination 2 (0%-60%), consumption of 0%-Splenda wet-mash was once again, much higher than that of 60%-Splenda wet-mash. Finally, in combination 3: (10%-60%), where both chows contained Splenda, rats were consuming much more of the 10%-Splenda chow, as compared to the 60%-Splenda chow. Just like Experiment 1, these results did not support the initial hypothesis where it was predicted that rats would prefer chows that were sweeter (containing more Splenda). Instead, the opposite occurred, where all subjects much preferred the 0%-Splenda chow over the ones containing Splenda. There was more evidence supporting this trend when differences in chow intake were compared within each of the 3 combinations. Referring back to Figure 9, the largest difference in chow intake between 2 Splenda concentrations was found within combination 2 (0%-60%). Thus, their preferred chow was 0%-Splenda, and their least preferred was 60%-Splenda. The smallest difference is intake was found in combination 1 (0%-10%). The 0%-Splenda chow was still preferred over the 10%-Splenda chow, but to lesser extent than the 60%-Splenda chow.

These results contradicted the hypotheses for this study and also what is found in some literature stating that rats generally prefer sweeter solutions over blander flavours, like water (Yeomans, 1998; Young, 1966;). The aversion to chows containing Splenda was consistent throughout both experiments for CNEs and CSEs, in both the stress and non-stress conditions. It was even more obvious when rats were given a choice between two different chows. The decrease in food intake in response to higher Splenda concentrations was also observed in Experiment 1. As discussed in Experiment 1, it was possible that the concentrations chosen for the experiments were too sweet, and thus became unpalatable to the subjects (Monneuse, et. al., 1991; Perez, et. al., 1994). As discussed in Experiment 1, the robust decrease in intake of chows containing Splenda could be due to the fact that the rats do not like sucralose, which is the main

ingredient in Splenda (Loney et al., 2011; Scalfani et al., 2004). This idea is being discussed in further detail within the limitations section.

Limitations and Future Directions

Rat Strain. A main limitation of this study was the animal strain that was chosen. Sprague-Dawleys were used for this study because they are a common strain used in research for animal models of learning due to their calm temperament. Although it was not one of our hypotheses, we became interested in exploring if these models of stress-eating and stress non-eating suggested by Johnson and Emond (2017), could be generalized to a different strain of rats. The study by Johnson and Emond (2017) used male Wistar rats to create their animal models of stress-eating and stress non-eating. Wistars have been found to be more hypervigilant and display more behavioural inhibition than Sprague Dawleys (McAuley, Stewart, Webber, Cromwell, Servatius, & Pang, 2009; Servatius, Jiao, Pang, & Minor, 2008). In humans, behavioural inhibition has been linked to increased sensitivity to stress, and increased reactivity of the HPA (Tyrka, Mello, Mello, Gagne, Grover, Anderson, Price & Carpenter, 2006). Due to these traits, Wistars make better animal models of anxiety and stress than Sprague-Dawleys. Thus, future research examining the phenomenon of stress-eating and stress non-eating, especially using a mild stressor such as a high-frequency tone, should be careful of strain choice for their experiment. Despite their similarities, Wistar rats make an ideal strain for this line of research, as compared to Sprague-Dawleys. However, if Sprague-Dawleys are to be used in future research, it would be advisable to use a more aversive stressor such as restraint or a mild foot shock.

Sweetener. A second limitation was the sweetening agent chosen for the palatability portion of the study. It became quite clear by the end of this study that on average, the rats

disliked chow containing Splenda (main ingredient sucralose). In other words, the more Splenda the chow contained, the less they would eat, despite being food deprived. Splenda was chosen to formulate sweet-tasting chows, without increasing the calorie content. However, rather than increase the palatability of these mixtures, it appeared to have the opposite effect. This is consistent with what was found in a study by Scalfani and Clare (2004), which looked at female Wistar rats' preferences between sweet solutions with various concentrations of saccharin or sucralose. Their findings were quite interesting; a bimodal trend was observed for sucralose where half of their subjects preferred sucralose over water, and the other half preferred water. A later study by Loney, et. al., (2011), labeled these rats as "sucralose preferrers" and "sucralose avoiders" and found sucralose preferrers represented 35% of their subjects, while sucralose avoiders represented 65% of their subject. Thus, this study demonstrated that more than half the rats were sucralose avoiders. Both studies suggested that in addition to being sweet in taste, sucralose has an off-taste to some of the rats. While most rats are very sensitive to this off-taste, others do not notice it at all (Loney et. al., 2011; Scalfani & Clare, 2004). In the current study, all of the rats consistently avoided chows containing Splenda, especially the one containing 60%-Splenda. Perhaps if a larger sample size was used, such as in the studies by Scalfani and Clare, (2004) and Loney et al., (2011) (n=50), there would have been some "sucralose preferrers" amongst them. As discussed earlier, Splenda was chosen as the sweetener for this study because it is a well-known non-caloric alternative to table sugar. It is becoming a very popular sweetening agent in processed foods, due to its similarities with sucrose, and its stability at various pH and temperatures (i.e. can be used in cooking) (Chattopadhyay, Raychaudhuri, & Chakraborty, 2014). Therefore, it was of value to look at its interaction (if any) with stress and eating.

Sample Size. Another limitation of this study might have been the small samples size. While the current study used 8-9 rats per group, for two experimental groups, the study by Johnson and Emond (2017) used 10 rats per group, for three experimental groups. In animal research, it is highly advised to use the least number of animals that is required, therefore, it is not uncommon to have smaller sample sizes. Since the Johnson and Emond (2017) study yielded promising results, and the current study was removing an experimental group, a decision was made to have 8-9 rats per group. Regardless, a slightly larger samples size (ex. 10 rats per group) might have allowed for the detection of additional significant differences, that were otherwise not found in the current study. Future research should definitely consider using a larger sample size, to increase power size and have better odds of detecting significant differences.

Future Directions. Future research may want to investigate stress-induced eating and non-eating with other sweeteners that are rapidly replacing sucrose in food and beverages, such as high-fructose corn syrup. High-fructose corn syrup is a caloric sweetener derived from processed corn. Due to its low costs of production, it has replaced the majority of caloric sweeteners in food and beverages (Bray, Nielson, & Popkin, 2004). High-fructose corn syrup is of interest because ever since its introduction, obesity in America has been rapidly increasing (Bray et al., 2004). It is believed that this is because high-fructose corn syrup does not get metabolized in the body as readily as sucrose, and as such gets stored in the body as fat (Bray et al., 2004). High-fructose corn syrup would be an ideal sweetener for future studies using an animal model of stress-eating and stress non-eating, because in normal circumstances (non-stress) it is highly palatable to rats at various concentrations (Colangelo et al., 2014). Additionally, it has strong rewarding properties (Levi et al., 2014), which means ingesting high-fructose corn syrup taps into the brain's reward system. As mentioned previously, palatable

foods that trigger the reward system in the brain, release endogenous opioids, which then suppresses HPA activity, terminating the stress response (Pecoraro et al., 2004).

Furthermore, it would be interesting to replicate a similar study with female rats, like the studies by Scalfani and Clare (2004) and Loney et al., (2011). The present study used male rats, because this is what has been used in past studies, and male and female rats are quite different from one another in terms of temperament, size, and their sensitivity to sweet-taste (Loney et. al. 2011). Most importantly, females go through estrus quite frequently which could have been a confound for the current study. Estrus produces notable physiological and hormonal changes in the female rat, to prepare her for a potential pregnancy. These changes can significantly affect both feeding behaviour and sensitivity to stress. Nonetheless, in a different study, their estrus cycle could be an interesting variable in the field of stress-eating and stress non-eating, and preference of palatable foods.

Implications

The results of this study were mostly unexpected and often opposite of what was initially predicted. Regardless, it adds useful information to current literature of stress, eating and palatability, in the following ways. For one, this is the first study to our knowledge that a) applied a modified version of the model of stress-eating and stress non-eating suggested by Johnson and Emond (2017) to examine the rats' preference of palatable food, and examine if changes in preference occur when the two groups are stressed, b) used Splenda (sucralose) as a tasteant within the scope of learned stress-eating and non-eating, and c) tested the animal model of stress-eating and stress non-eating with a different strain of rat, to see if this model could be generalized to other strains of rats.

Although subjects did not show a preference for sweeter foods under stress, which might have been a factor of the chosen tasteant itself, feeding behaviour did change for one of the groups in the presence of the high-frequency tone. Conditioned stress-eaters, which learned that eating would result in the removal of the stressor, increased their overall food intake when the stressor was present, as compared to when it was absent in the second experiment.

However, this was not observed for conditioned stress non-eaters, possibly because their drive to satisfy their hunger, competed with the stress-response. Since hunger is a very strong driver of behaviour, as it is a basic living need, this group was likely more concerned with replenishing their energy needs, than the removal of the stressor. This is probably because starvation is more of a threat to their survival, compared to a loud noise. On the other hand, conditioned stress-eaters did not have this issue because satisfying their hunger and removing the stressor, were one in the same. These findings could help us better understand stress-eating behaviour, in an animal that typically reacts to stress by limiting their food intake. It is evident from our results that conditioned stress-eaters are prone over-eating when presented even with a mild stressor such as a high-frequency tone. Since increased food intake also means increased calorie intake, in time, this behaviour could produce significant weight gain. In turn, this could lead to a more severe problem such as obesity. The information uncovered in this study could be applied to humans, to help stress-eaters find healthier coping mechanisms such as exercise or meditation.

Additionally, the main results of this study gave some insights on how to further improve the animal model of stress-eating and stress non-eating as a function of differing learning histories. Most importantly, this study revealed that both, strain choice and reward-pellet chosen are very important components to consider for creating a successful animal model of stress-eating and

stress non-eating. Future studies should aim to use a rat strain that is more sensitive to stress, such as Wistars, which were originally used in the Johnson and Emond (2017) study. Additionally, precision-pellets containing sucrose, unlike the bland ones used in this study, may be more effective in rewarding the desired behaviours during training. Furthermore, it was clearly demonstrated in this study, that the subjects did not find Splenda to be palatable. Regardless, it was of value to examine this tasteant within the scope of stress-eating and non-eating due to its increasing presence in human food and beverages.

Conclusion

The present study looked at different learning histories as a factor contributing to stress-eating and stress non-eating in humans. Operant conditioning was used to model stress-eating (negative reinforcement) and stress non-eating (punishment training) in rats. This model was then used to explore these groups' preferences for food containing different amounts of Splenda, in the presence and absence of a mild stressor. Overall, the results found in this study provided some evidence for past learning histories as a contributing factor of stress-eating, but not of stress non-eating. CSEs, who learned to associate feeding with the removal of a stressor, increased their overall food intake in the presence of a stressor, as compared to when it was absent. However, CNEs kept their food intake similar regardless of the condition. These findings suggested that CSEs could more vulnerable than CNEs to changes in feeding behaviour in response of stress. The implications are that, over-eating in general, also means an increase in calorie intake. Thus, if an individual is prone to over-eat in response stress, this could lead to problematic weight gain. Further exploration of factors contributing to learned stress-eating could help treat these individuals, by creating training programs that extinguish the maladaptive

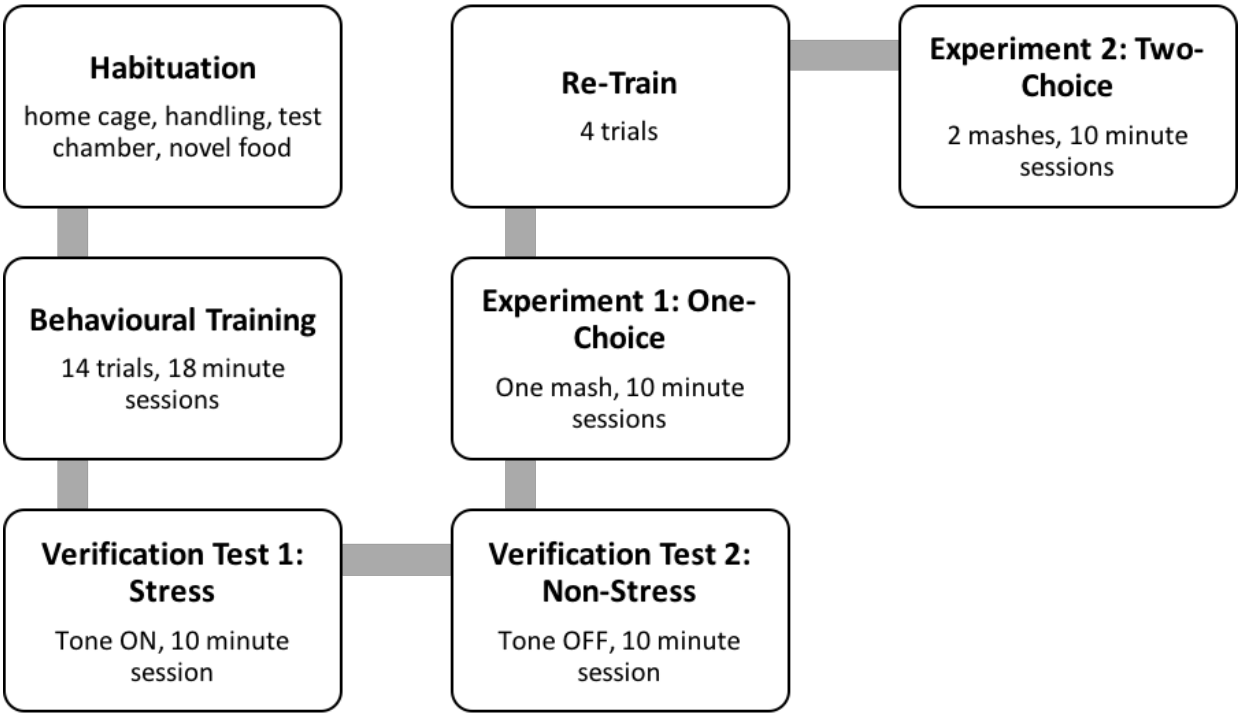
feeding behaviour, in response to stress. Additionally, it could help these individuals find better coping mechanisms when faced with stressful situations.

With respect to the palatability component of the study, it was found that all subjects preferred chow containing 0%-Splenda, over the other two concentrations (10% and 60%), regardless of whether or not a stressor was present. Thus, contrary to initial predictions made for this study, all subjects preferred the blander chow, and this preference was not affected by stress. These findings were interpreted as possible issues of tasteant choice and tasteant concentrations. Despite its growing popularity in human food and beverages, the subjects in this study showed a strong aversion to chows containing Splenda. This suggested that Splenda may have been too sweet and/or had bad-tasting properties, compromising the palatability of this sweetener.

The present study added to the current literature by providing important information to consider in the making of a reliable animal model of stress-eating and stress non-eating, as a factor of different learning histories. Choice of appropriate animal strain has shown to be an essential feature of this model. Future studies should use a strain that is more sensitive to stress, such as the Wistars used in Johnson and Emond (2017). Overall, stress, eating, and palatability are complex mechanisms. The current study only scratched the surface, and there is still a lot of information to be uncovered within this field of research. Future directions should aim in using this model in female rats, and also explore preferences for other common sweetening agents found in food and beverages, such as high-fructose corn syrup.

APPENDIX A

Timeline of Methods:



APPENDIX B

Copies of ACC Ethics Approval Letters:

LaurentianUniversity
UniversitéLaurentienne

TO: Dr. Michael Emond – Department of Psychology

FROM: Dr. David MacLean, Chair of the Animal Care Committee

DATE: April 15, 2016

RE: AUP modification 2013-06-03 – The effects of negative reinforcement and punishment training on eating rats

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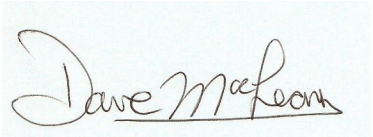
Dr. Emond,

On April 13, 2016, the Animal Care Committee met to consider the above-mentioned modification to an existing protocol and voted to approve it.

If you need further information, do not hesitate to contact me.



David MacLean, PhD.
Chair of the Animal Care Committee

LaurentianUniversity
UniversitéLaurentienne

TO: Dr. Michael Emond

FROM: Dr. David MacLean, Chair of the Animal Care Committee

DATE: October 13, 2016

RE: Renewal 2013-06-03 – The effects of negative reinforcement and punishment training on eating in rats

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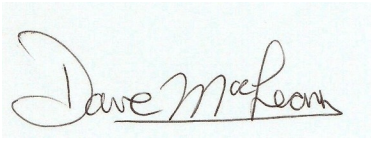
Dr. Emond,

On October 12, 2016, the Animal Care Committee met to consider the above animal use protocol renewal. Since we did not have quorum at the meeting, the protocol was approved by the interim committee and will be brought forward to the next scheduled meeting on November for formal approval. Until then, please consider this letter your approval to begin your studies.

The Canadian Council for Animal Care (CCAC) has updated its recommendations for Terms of Reference for local ACC's. One of these recommendations requires an increase in the monitoring of AUP's by the ACC after approval. Thus, a new (and short) form must now be filled out by all Principal Investigators 6 months after the protocol is initiated and renewed. This new form is available on the university website.

If you need further information, do not hesitate to contact me.



David MacLean, PhD.
Chair of the Animal Care Committee

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