The Effects of Learning on the Eating Responses of Rats Exposed to Stress

by

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Abstract

Literature examining the relationship between stress and eating behaviour reveals that while both decreased and increased eating are observed in response to stress, it is unclear what factors determine whether an individual will typically decrease or increase eating during stress. The present study sought to explore whether decreased/increased eating in response to future stressors, could be elicited by prior associations between decreased/increased eating and the presentation of a stressor, through operant conditioning. The "Conditioned Non-Eaters" (CNE) group received punishment training while the "Conditioned Eaters" (CE) group received negative reinforcement training in response to a noise stressor, and the Control group received no operant training. Conditioning trials were followed by a series of five tests exposing subjects to no stress, the noise stimulus, threat of shock, restraint, and a tail-pinch. Results indicate that group differences in eating were observed during exposure to the noise stimulus following training, but not in the absence of stress, and consistent group differences to the noise stress were further observed during exposure to novel stressors. These findings suggest that past operant associations between eating and a specific stressor can generalize to other stressors, influencing individuals to respond to stress exposure in a way that has be reinforced in the past.

Keywords: stress, eating behaviour, operant conditioning

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In human populations, it is recognized that individuals have a tendency to either overeat or undereat when experiencing stress, however reasons for this divergence in the population remain unexplained. In both human and animal populations, current literature relating to the effects of stress on feeding behaviour present various and contradictory findings (Greeno & Wing, 1994). Thus, it is widely accepted that aversive stimulation or stress interrupts normal feeding patterns and can have a bi-directional impact on the eating response of organisms, however, whether stress will cause an individual to increase or decrease their eating has not proven to be easily predictable (Greeno & Wing, 1994). In humans, it is suggested that approximately 40% of individuals report increased eating in response to stress, 40% report decreased eating, while the remaining 20% of individuals report no changes in eating behaviour during stress (Dallman, 2010).

The present study seeks to examine a possible cause for this bi-directionality – specifically that differences in past learning may be a factor in determining which of these behaviours will be experienced by individuals in response to stress during future situations. In an attempt to support this theory, the present research explores the influence of individual differences in learning history on the feeding response of rats in response to stress, using an operant learning paradigm. By employing negative reinforcement and punishment training, the present study sought to condition subjects to increase or decrease their eating in the presence of a stressor (in this case an aversive noise stimulus). The study further tested the feeding response of individuals following conditioning, when presented with novel stressors (with which subjects had no prior exposure to), to determine whether learned changes in eating behaviour will generalize to other types of stressful stimuli.

Stress

To understand how past associations between eating and stress may shape future eating behaviour, it is important to understand the biological changes involved in the stress response that include hormone and neurotransmitter changes that influence the feeding system in various ways. Furthermore, if learned associations between stress and changes in eating behaviour can generalize to future experiences of stress as predicted by the current model, an understanding of how stress affects the body and feeding system may contribute to identifying what properties of the stress response may be involved in becoming associated with these behaviours. A review of past literature reveals that researchers have been able to reach a collective understanding of what defines stress (Staal, 2004). While proposed definitions range widely between authors and are often conflicting, they frequently relate to an organism's perception of and ability to cope with threatening stimuli (Adam & Epel, 2007; Cohen, Janicki-Deverts, & Miller, 2007; Greeno & Wing, 1994; Pool, Brosch, Delplanque, & Sander, 2015; Staal, 2004). Stress has further been defined as a somato-psychic state that represents a deviation from the optimal level of the organism (Chrousos & Gold, 1992; Krebs, Macht, Weyers, Weijers, & Janke, 1996). Homeostatic mechanisms, in turn, motivate behaviour to return the organism to its optimal level of activation (Chrousos & Gold, 1992; Krebs et al., 1996).

Stress engages the "fight-or-flight" reaction, having evolved as a survival response allowing organisms to allocate their energy towards reacting to life-threatening stimuli or situations as first described by Walter Cannon in 1932. When a stressor is presented, the amygdala communicates a distress signal to the hypothalamus, stimulating the adrenal glands to release epinephrine. This leads to an increase in various physiological responces controlled by the sympathetic nervous system, including heart rate, blood pressure, and breathing rate (Tsigos

& Chrousos, 2002). The parasympathetic nervous system responds with a reduction of activity during stress, resulting in decreased blood flow to the digestive system, as digestion and other bodily processes are reduced allowing energy stores to be allocated towards responding to the threat or stressor (Petri & Govern, 2004).

Activation of the hypothalamic-pituitary-axis (HPA) stress response is characterized by hypothalamic release of corticotropin-releasing hormone (CRH), which stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, further promoting the adrenal release of glucocorticoids to the bloodstream (Majzoub, 2006). Cortisol release may continue for hours following a stressor and homeostasis returns following high blood concentrations of cortisol that stimulate negative feedback to the hypothalamus, reducing the release of CRH and consequently the release of ACTH from the pituitary, diminishing the HPA stress response (Majzoub, 2006). Increased intensity of a stressor can be evaluated by increased activation of the HPA axis, increased blood cortisol levels, and an increased post-stress recovery period for both rats (Samson, Sheeladevi, Ravindran, & Senthilvelan, 2007) and humans (Dickerson & Kemeny, 2004). Therefore, increased hypothalamic CRH, peripheral cortisol, and ACTH levels are all physiological markers of stress (Adam & Epel, 2007; Dickerson & Kemeny, 2004; Maniam & Morris, 2012; Tsigos & Chrousos, 2002).

The HPA response to stress has further been shown to persist even following chronic (long-term) exposure to a stressor (Samson et al., 2007). In this study, noise stress continued to result in increased release of corticosterone and norepinephrine in rats even following chronic exposure to the noise stressor (Samson et al., 2007). Adaptation of the HPA response to a specific stressor has however been demonstrated; in a study investigating the effects of stress on the response of anterior pituitary hormones in rats also subjected to chronic noise stress, rats

demonstrated a reduced corticosterone response when presented with the same noise stressor following the chronic stress, though this adaptation of the stress response was identified as specific to the noise stressor and did not generalize to a forced swim test (Armario, Lopez-Calderon, Jolin, & Balasch, 1986).

Chronic stress may have long-term implications on physical and psychological health. Research reveals that repeated stress may contribute to future physical health problems such as high blood pressure and clogged arteries, and has also been implicated in psychological disorders such as anxiety, depression and addiction (Dallman, 2010; Parylak, Koob, & Zorrilla, 2011; Tsigos & Chrousos, 2006). It has further been proposed that stress may contribute to obesity directly, by causing changes in food intake patterns, or indirectly, by reducing rate of rest and exercise. Research on stress and obesity raise questions regarding the effects of stress on the feeding system, while research on stress, addiction, and the hedonic (pleasurable) properties of foods raise questions regarding the interactions between stress and neural reward pathways (Dallman, 2010; Dallman et al., 2006). Before delving into a review of past human and animal research investigating the effects of stress on feeding behaviour, it is important to review how the stress, feeding, and reward systems interact.

Stress and the Feeding System

The physiological mechanisms involved in eating motivation include both short-term and long-term hunger perception. When nutrient levels in the bloodstream are low, the hypothalamus relies on hunger signals from receptors in the liver, stomach, and small intestine for short-term hunger perception, while long-term mechanisms involve the hypothalamus monitoring reservoirs of fat in adipose tissues for the secretion of peptide hormones indicating that they are full (Petri & Govern, 2004). These hormones inhibit brain mechanisms involved in the control of eating

when they are high, causing the hypothalamus to be less sensitive to short-term hunger signals (Petri & Govern, 2004).

While the mechanisms underlying hunger motivation are still not entirely understood, several hormones and neurotransmitters involved in the stress response have also been implicated as regulators of hunger motivation and feeding behaviour. Cholecystokinin (CCK) is a hormone secreted by the upper intestine following eating and its receptors send satiety signals to the brain as a short-term regulator of food intake, inhibiting eating behaviour when levels are high (Petri & Govern, 2004). Further research has implicated CCK as involved in the experience of anxiety (Maniam & Morris, 2012). Another hormone, leptin, is released into the bloodstream by adipose cells to signal information about the amount of energy available in fat stores for long-term hunger perception to the arcuate nucleus (ARC) of the hypothalamus (Cavagnini, Croci, Putignano, Petroni, & Invitti, 2000). Insulin levels in the blood further communicate information to the hypothalamus regarding blood glucose levels and energy available in fat stores to control long-term feeding behaviour (Petri & Govern, 2004). Both leptin and insulin levels increase when glucocorticoid levels are elevated during stress. Notably, in studies investigating elevated glucocorticoid activity in response to chronic stress have suggested that increased stress may result a reduction of hunger through this interaction (Adam & Epel, 2007).

Furthermore, neuropeptide Y (NPY) is a neurotransmitter secreted by the ARC in response to low leptin and insulin levels; acting on the paraventricular nucleus (PVN) of the hypothalamus, it promotes feeding and reduces energy expenditure (Hanson & Dallman, 1995). Likewise, alpha-melanocyte-stimulating hormone (α-MSH) is secreted in response to high leptin and insulin levels and consequently reduces feeding behaviour (Fehm, Born, & Peters, 2004). NPY levels elevate with increased release of glucocorticoid released during stress, possibly

increasing hunger, while α-MSH is believed to be involved in the stress response as ARC neurons expressing this hormone project to the HPA axis, suggesting that increased eating during stress may be further mediated by the melanocortin system (Fehm et al., 2004).

CRH also influences both the stress and feeding responses as it acts on two types of hypothalamic receptors to induce the stress response and to suppress feeding behaviour (Majzoub, 2006). Finally, ghrelin (GH) is a peptide secreted by the stomach believed to be involved in short-term regulation of hunger, as it binds to receptors in the hypothalamus to initiate feeding behaviour and is further known to inhibit leptin's action on the hypothalamus (Seoane, Al-Massadi, Lage, Dieguez, & Casanueva, 2004). GH promotes feeding and GH may be a further mediator of the effects of stressor type on eating behaviour, as GH levels increase in response to various types of stressors but have also been shown to decrease in response to isolation stress (Lutter et al., 2008; Saegusa et al., 2011). Important to note is the role of the hypothalamus in initiating both feeding behaviour and the stress response; these two physiological responses share neural circuitry and can influence each other (Adam & Epel, 2007). While the directional effects of stress on feeding behaviour are still not well understood, they are likely mediated through interactions between glucocorticoids with hormones and neurotransmitters known to affect feeding behaviour due to the overlapping neural biology of the stress and feeding systems.

Stress and the Reward System

Important to the present research is a discussion of the reward systems' interaction with the stress response and its involvement in motivating feeding behaviour. The mesolimbic system is the brain's reward pathway and is comprised of dopaminergic neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens (Petri & Govern, 2004). Dopamine

levels increase when this system is activated, signalling the value of a rewarding stimulus, making this system responsible for incentive-driven motivated behaviour (Cota, Tschöp, Horvath, & Levine, 2006). The reward system interacts with the amygdala and hippocampus to establish memories of rewarding stimuli and behaviours, increasing future motivation to obtain rewards such as the example of future drug-seeking behaviour in the case of addiction (Cota et al., 2006).

The stress and reward systems interact directly as CRH is released to the VTA in response to acute (short-term) stress, however, while CRH is known to suppress both feeding and stress responses, the reward system is known to interact specifically with responses to highly palatable foods (Cavagnini et al., 2000). High food palatability is frequently associated with food that is high in calories and fat, and that is sweet tasting, though palatability is determined primarily by the hedonic properties of a food (Yeomans, 1998). Researchers have compared the hedonic properties of highly palatable foods to drugs of dependency and support the idea that these foods may have properties that promote dependence by activating reward circuitry, promoting behavioural reinforcement for their increased consumption (Cota et al., 2006).

The role of the opioid system in the rewarding aspect of highly palatable foods has been explored as opioid antagonists have been shown to decrease the consumption of foods with hedonic properties (Cota et al., 2006). The opioid antagonist naltrexone, has been found to decrease the rewarding properties of sucrose in both animal and human populations and naloxone has reduced stress-induced eating in rats, implying that this system is likely further involved in the rewarding aspects of palatable foods and may play a role in overeating behaviour (Cota et al., 2006; Morley, Levine, Gosnell, & Billington, 1984; Morley, Levine, Yim, & Lowy,

1983). Opioids have further been found to result in a decreased stress response (Drolet et al., 2001).

The endocannabinoid system is further believed to interact with the opioid system. Cannabinoids have previously been associated with increased consumption of palatable foods and in one study involving rats, opioid antagonists were found to reverse increased consumption of a palatable beverage that had been caused by previous administration of a cannabinoid agonist (Gallate, Saharov, Mallet, & McGregor, 1999). While research suggests that opioid and cannabinoid systems may be involved with increased or decreased eating, the role of the reward system in mediating the effects of stress and the consumption of palatable foods are still not well understood.

The Effects of Stress on Feeding Behaviour

In rat studies, stress has been most often associated with decreases in food intake, though in some cases it has been associated with increased feeding behaviour (Greeno & Wing, 1994). Recent research has also suggested that stress is more likely to specifically increase the consumption of highly palatable foods, with the consumption of lard and high sucrose solutions leading to a reduced stress response in rats (la Fleur, Houshyar, Roy, & Dallman, 2005; Pecoraro, Reyes, Gomez, Bhargava, & Dallman, 2004). The bi-directional effects of stress on eating behaviour in humans have also been widely studied, however the causes of overeating and undereating in response to stress are still not well understood (Adam & Epel, 2007; Greeno & Wing, 1994; Pool et al., 2015). Several theories have proposed explanations for both overeating and undereating by animals and humans during stress and it is clear that there are likely multiple factors that will determine an individual's feeding in response to stress (Greeno & Wing, 1994).

The general effect model. Attempts to explore the bi-directionality of eating in response to stress have investigated a general effect model, one suggesting that aversive stimulation will have a unidirectional effect of decreasing eating behaviour (Greeno & Wing, 1994). The general effect model of reduced eating during stress is founded on increased HPA activation during stress. A purely physiological perspective would propose that appetite should be reduced during aversive stimulation due to a reduction of energy allocated to digestive processes (Greeno & Wing, 1994). However, attempts to support this theory have been unsuccessful as researchers exploring this model using various paradigms in animals and humans have reported conflicting data (Greeno & Wing, 1994).

Animal research. The general effect model has been investigated in animals extensively in laboratory studies using various types of stressors. Restraint stress has resulted in decreased feeding behaviour in rats fairly consistently (Calvez et al., 2011; Martí, Martí, & Armario, 1994; Rybkin et al., 1997). Immobilization has had the same effect (Martí et al., 1994; Vallès, Martí, & Armario, 2003), as have forced swim tests (Calvez et al., 2011), while cold water swim tests have resulted in increased feeding behaviour (Vaswani, Tejwani, & Mousa, 1983). Social defeat has resulted in decreased (Solomon, Karom, & Huhman, 2007) and increased (Campbell Teskey, Kavaliers, & Hirst, 1984; Foster et al., 2009) feeding behaviour in different studies. Conflicting data has also been reported for the effects of mild tail-pinch stress as rats have demonstrated an increased feeding response to this stressor (Antelman & Szechtman, 1975; Dess, 1997; Levine & Morley, 1981, 1982; Rowland & Antelman, 1976), while in other research it has resulted in no effect on feeding behaviour (Meadows, Phillips, & Davey, 1988). Shock stressors have resulted in decreased feeding in animals (Solomon et al., 2007; Sterritt, n.d.; Tugendhat, 1960), increased feeding (Siegel & Brantley, 1951; Ullman, 1951, 1952), and they have also had no effect on

feeding responses (Sterritt, 1965; Strongman, 1965). Furthermore, the feeding response of rats has decreased in response to chronic noise (Alario, Gamallo, Beato, & Trancho, 1987) but increased following acute noise (Kupfkrmann, 1964). Chronic stress has further led to increased consumption of palatable foods in primates, where social subordinance was the examined stressor (Wilson et al., 2008).

Inconsistencies in the effects of various stressor types on the feeding behaviour of animals have encouraged researchers to investigate what qualities of stress may predict how feeding behaviour will be affected, though these remain unclear. Comparisons between the effects of stressors varying in intensity have indicated a correlation between stressor intensity and the magnitude of changes in feeding behaviour observed, for example, in a comparison of chronic handling, restraint, and immobilization stress on the feeding behaviour of rats, immobilization resulted in the greatest reduction of feeding behaviour, followed by restraint and handling (Martí et al., 1994). Overall, there is a consensus that acute stress most often results in reduced eating by animals and most instances of increased eating in response to stress have been attributed to emotional stressors or have been selective to foods with hedonic (Maniam & Morris, 2012).

Human research. Human investigations of the general effect model have less frequently examined laboratory-induced stressors and often rely on self-report of subjective stress experienced by participants. As with animal studies, a pattern of decreased eating during stress has been evidenced, with exceptions with regard to highly palatable foods though not all evidence supports this (Groesz et al., 2012). In one study, subjective daily records of stress and feeding behaviour were recorded and both within and between-subjects analyses indicated that individuals were more likely to eat less during increased stress and,furthermore, that the

likelihood of decreased eating correlated with increased stressor severity (Stone & Brownell, 1994). Combat stress was associated with decreased eating in a survey of U.S. Marines (Popper, Smits, Meiselman, & Hirsch, 1989) and in another study, males were found to eat less in response to a mild laboratory stressor (ego threat) while no differences were observed for women, suggesting that gender may influence these effects (Grunberg & Straub, 1992).

The general effect model is challenged by human studies as it is well-evidenced that some individuals self-report being stress non-eaters while others report being stress eaters or report no change in eating behaviour during stress (Dallman, 2010). While exam stress caused no significant effects on food intake in one study (Pollard, Steptoe, Canaan, Davies, & Wardle, 1995) and produced inconsistent findings in another (Michaud et al., 1990), a study comparing exam stress between self-reported stress eaters and non-eaters revealed that increased feeding behaviour (weight gain) during stress was observed for the stress-eaters group (Epel et al., 2004). Human studies suggest that while stress may have bi-directional effects on feeding behaviour, individuals tend to respond consistently to stress resulting in distinct populations of stress eaters and stress non-eaters (Wardle, Steptoe, Oliver, & Lipsey, 2000). This is supported by evidence that individuals in these groups demonstrate further differences in their stress response, as stress eaters have been found to have higher nocturnal levels of insulin and cortisol during stress compared to stress non-eaters (Epel et al., 2004).

Summary. While physiological implications of the stress response led to the hypothesis that activation of the stress response should lead to decreased feeding behaviour and digestive processes, this has not been evidenced consistently (Greeno & Wing, 1994). Stress can elicit overeating in some individuals while eliciting undereating in others, promoting further research

to investigate what other factors affecting the feeding and stress responses may be responsible for divergent populations of stress eaters and non-eaters.

Individual differences. As a unidirectional model of the effects of stress on feeding behaviour has not been supported by research, it is important to consider that individual differences may be key to understanding the cause for different eating responses between individuals in response to identical stressors (Greeno & Wing, 1994). The individual differences model suggests that differences in past experiences, attitudes, or physiology may determine how stress will affect an individual's feeding response to stress (Greeno & Wing, 1994). Individual differences between obese and normal weight individuals, between restrained and unrestrained eaters, and between individuals with high and low cortisol reactivity have been investigated, yielding further insight but also further inconsistent findings between studies of human and animal populations (Greeno & Wing, 1994) .

Restrained versus unrestrained. Restrained eaters (individuals with a history of attempting to chronically restrain their eating) have been compared to unrestrained eaters (individuals without a history of dieting) with regard to how these populations differ in their feeding response to stress (Greeno & Wing, 1994). Despite some conflicting data, many researchers believe that a history of dieting is a causal factor of stress induced feeding behaviour. Restrained eaters have shown to be more likely to overeat when faced with stressful experiences causing an unpleasant emotional state and it is proposed that this may be due to the control they usually attempt to maintain over their eating being interrupted by unpleasant emotional states (Willenbring, Levine, & Morley, 1986).

Animal and human research. Investigations of the influence of dietary restraint on the stress-eating relationship have been mostly provided by human studies. Baucom & Aiken (1981)

demonstrated that dieting subjects were more likely to increase feeding in the face of stressful conditions, while non-dieting subjects were more likely to decrease their eating response. Food intake of female restrained and unrestrained eaters has been assessed following various laboratory-induced stressors and restrained eaters have demonstrated increased eating following a reaction time stressor compared to decreased eating during a relaxation period (Lattimore & Caswell, 2004). Restrained eaters have further demonstrated increased feeding behaviours in response to stress induced by a film (Cools, Schotte, & McNally, 1992; Schotte, Cools, & McNally, 1990).

Similarly, the influence of dietary restraint on eating in response to stress has been investigated outside of laboratory settings; restrained eaters are more likely than unrestrained eaters to report being stress eaters (Wardle et al., 2000). Furthermore, in a sample of adult employees of a department store, high work stress periods led to increased caloric intake of saturated fat and sugar, an effect that was significant only for restrained eaters (Wallis & Hetherington, 2009). Animal studies have also supported these findings as rats have demonstrated increased saccharine preference in response to shock, only when rats had been subjected to a history of dietary restriction. Herman & Polivy (1975), identified a three-way interaction between reported anxiety, food deprivation, and dietary restraint; for food deprived, unrestrained eaters, anxiety suppressed hunger, while it increased eating for food-deprived restrained eaters.

Contrary to support for stress-induced feeding promoted by a history of dietary restriction, there has been no observed interaction between dietary restraint and exam stress (Pollard et al., 1995) and in another study, an ego-threat task resulted in decreased consumption of low-fat foods by restrained eaters, while differences in eating behaviour were not found with

regard to the consumption of highly palatable foods (Wallis & Hetherington, 2009). Increased consumption of high-fat foods have also been correlated with emotional but not with restrained eating (Wardle et al., 2000).

Obese versus normal-weight. Further investigation of individual differences in eating behaviour have compared the effects of stress on obese compared to normal weight individuals (Greeno & Wing, 1994). Obesity rates are increasing in our society and it has been proposed that increased feeding behaviour by obese individuals during stress compared to normal weight individuals may be a factor contributing to the development and maintenance of obesity (Dallman et al., 2006). Early research comparing obese and normal weight populations supported this, suggesting that overweight individuals are more likely than normal weight individuals to overeat when faced with environmental stressors (Stunkard, 1959). However again, animal and human studies have resulted in inconsistent findings with regard to this theory.

Animal and human research. Research examining the effects of obesity on the stress-eating relationship has again relied most frequently on human studies. Exam stress resulted in increased consumption of candy by obese individuals, with no change in eating behaviour reported for normal-weight individuals (Slochower, Kaplan, & Mann, 1981) and threat of shock has resulted in consistent findings for the consumption of peanuts, though weight was not corrected for in this study (Pine, 1985). There was no change in the consumption of chocolate for either group in an experiment where performance was linked to shock (Reznick & Balch, 1977), while another study found that obese individuals decreased their consumption of ice cream following an ego-threat stressor, with no change in feeding behaviour by normal weight individuals (Heatherton, Peter, & Polivy, 1991).

Animal studies have failed to investigate the influence of obesity on the stress-eating relationship, however stress-induced eating of palatable foods has resulted in weight gain by rats in some studies (Rowland & Antelman, 1976). These findings suggest that while the effects of obesity on the stress-eating relationship is unclear, stress-induced eating may contribute to weight gain and resulting obesity.

It is likely that other variables may interact with obesity to affect feeding behaviour, for example, obese individuals with binge eating disorder may exhibit a different response to stress than obese individuals who do not have binge eating disorder and individuals with bulimia nervosa, demonstrated by an increased average eating rate caused by stress induced by the Trier Social Stress Test (Laessle & Schulz, 2009). Stress-induced eating also does not consistently result in weight gain in animals and weight gain may be dependent on the consumption of highly palatable foods (Levine & Morley, 1981). Gender differences may also be a factor, as normal-weight males demonstrated increased feeding when calm compared to frightened and more when food deprived than not, however obese males did not alter their eating behaviour to these variables and demonstrated similar feeding behaviour whether or not they were frightened or food deprived (Schachter, Goldman, & Gordon, 1968).

High versus low stress reactivity. High stress reactivity has further been investigated as a possible cause of increased eating during stress (Adam & Epel, 2007; Newman, O'Connor, & Conner, 2007). Elevated cortisol levels during stress have resulted in increased caloric intake, as evidenced by individuals taking prednisone (Wanefried, Rimer, & Winer, 1997). Individuals with high cortisol reactivity have demonstrated increased caloric intake in response to stress in both human and animal research (Newman et al., 2007). Psychological stress reactivity has

therefore been identified as a possible psychobiological characteristic explaining divergent feeding responses to stressors.

Animal and human research. Support for the effects of cortisol reactivity on the stress-eating relationship is offered by both human and animal studies. In a laboratory study using ego threat as a stressor, women with high cortisol reactivity consumed more calories than women with low cortisol reactivity during stress, while eating similar amounts in the absence of stress (Epel, Lapidus, McEwen, & Brownell, 2001). Similar findings have been reproduced by other researchers as individuals with high trait anxiety have also reported increased consumption of palatable foods during stress, compared to controls (Rutters, Nieuwenhuizen, Lemmens, Born, & Westerterp-Plantenga, 2009). In naturalistic settings these effects have persisted as high cortisol reactors self-report increased consumption of snack foods during periods of increased daily stress (Newman et al., 2007).

Support for the influence of individual cortisol reactivity on the stress-eating response is further offered by findings that individuals who self-identify as stress eaters display higher levels of cortisol in response to stress than stress non-eaters (Epel et al., 2004). Animal research has further revealed that increased corticosterone levels through the administration of glucocorticoids, has resulted in increased feeding behaviour in rats (Bhatnagar et al., 2000).

Summary. Investigations of the influence of individual differences on the stress-eating relationship have identified multiple factors that likely interact with the effects of stress on feeding behaviour. While obesity has not consistently resulted in increased eating in response to stress, a history of dietary restraint and high cortisol reactivity may be significant factors involved in producing this response. However, it is important to note that increased feeding behaviour during stress has most often been observed with regard to highly palatable foods

specifically (Adam & Epel, 2007). This suggests that the hedonic properties of foods are likely a significant factor leading to increased eating in response to stress and as a result, the implications of food palatability on the stress-feeding interaction have been a focus of recent research.

Implications of Food Palatability

With highly palatable food being so readily accessible in our Western society, changes in eating behaviour, specifically with regard to highly palatable foods during stress, is a proposed reason for the high percentage of individuals who self-report being stress eaters (Adam & Epel, 2007). As previously discussed, the general effect model for decreased eating in response to stress has been supported by many studies demonstrating stress-induced eating inhibition, though in experiments where highly palatable food is made available during stress, eating behaviour may alternatively increase (Adam & Epel, 2007; Dallman et al., 2006; Pecoraro et al., 2004; Pool et al., 2015; Tomiyama, Dallman, & Epel, 2011). Theories that stress may result in an increased willingness to consume sweet foods are supported by the fact that corticosterone replacement in adrenalectomized rats restores their willingness to drink sweet (saccharin) solutions (Bhatnagar et al., 2000; Dallman et al., 2006). Increased consumption of highly palatable foods during stress has been well evidenced in human and animal populations and has been attributed to attempts at emotional regulation and to activation of the brain's reward circuitry (Pool et al., 2015).

Emotional Regulation. A proposed explanation for increased consumption of highly palatable foods during stress is the aversive state reduction hypothesis, a theory proposing that the aversive emotional state experienced during stress is reduced following the consumption of highly palatable foods because their hedonic properties elicit an opposing pleasurable experience (Pool et al., 2015). Evidence of increased preference for palatable foods following emotional stress has been supported by research with evidence of the ability of palatable foods to result in a

reduced HPA response in animals and humans. Chronic stress is thought to increase expression of CRH in the amygdala due to chronically high levels of glucocorticoids. As elevated glucocorticoids promote the consumption of palatable foods, this promotes the ingestion of "comfort foods" to reduce the effects of chronic stress in the nucleus accumbens (Dallman et al., 2003; Dallman, Pecoraro, & la Fleur, 2005; Drolet et al., 2001). In studies exploring this hypothesis, restraint stress in animals has been frequently equated to emotional stress experienced by humans with some researchers suggesting this is because of greater involvement of the amygdala during restraint stress compared to other stressors (Dayas, Buller, Crane, Xu, & Day, 2001).

Animal research. The aversive state reduction hypothesis is supported by evidence that restraint stress increases preference for highly palatable foods and that access to these foods results in a reduced HPA response to both acute and chronic emotional stress in rats. In an early experiment, foot shock and restraint were used to compare physical and emotional stress in rats; while physical stress reduced saccharin preference, emotional stress increased its consumption compared to water (Alario et al., 1987). Physical stress has furthermore resulted in long-term decreased saccharin preference and decreased open field activity in an elevated plus maze, while emotional stress caused these behaviours to slightly increase (Pijlman, Wolterink, & Van Ree, 2003).

Reduced corticosterone release in response to restraint stress has further been demonstrated in rats receiving prior access to highly palatable foods (Kinzig, Hargrave, & Honors, 2008). However, in a study where rats were fed diets of either regular chow, a lard/chow mix, or a choice of lard and chow, a reduced HPA response was only observed in response to restraint stress in the choice group, which may be relevant to understanding the increased

consumption of palatable foods by humans who generally have control over choice of the types of food they consume (la Fleur et al., 2005). In another study, acute restraint stress was found to have no effect on the consumption of a highly palatable food (fruit loops) while chronic restraint stress resulted in increased consumption of this food (Ely et al., 1997). Furthermore, comparing chronically restrained rats to unstressed controls resulted in increased consumption of comfort foods by stressed rats and further demonstrated that CRH levels were reduced in both groups, while ACTH responses were diminished in restrained rats offered comfort foods (Pecoraro et al., 2004).

Human research. In humans, evidence of emotional regulation through feeding has been suggested by correlations between increased emotional stress and increased eating behaviour (Macht, Haupt, & Ellgring, 2005). Similar findings have been reported for highly palatable foods specifically; in one study, eating highly palatable chocolate was associated with emotional eating and was found to reduce negative mood for an immediate but short period of time, while having no effect on neutral or positive mood states (Macht & Mueller, 2007). Furthermore, a reported high-stress group of women reported more emotional eating and displayed higher mesenteric fat and BMIs than controls (Tomiyama et al., 2011). The same study provided evidence that HPA response may be diminished by the consumption of comfort foods as the high-stress group further demonstrated a diminished cortisol response following exposure to an acute laboratory stressor (Tomiyama et al., 2011).

Summary. The aversive state reduction hypothesis has been supported by both animal and human studies demonstrating that emotional stress may lead to increased consumption of palatable foods and a reduced stress response. Contradictory findings have however been reported and this theory is criticized for failing to account for all examples of stress-induced

eating of palatable foods. It has further been criticized that stress inhibits the experience of hedonic pleasure as stress may reduce taste perception of sweet foods in humans and can reduce consumption of sweet foods in rats (Al'Absi, Nakajima, Hooker, Wittmers, & Cragin, 2012; Enkel, Spanagel, Vollmayr, & Schneider, 2010). Additionally, it is unclear what aspects of food palatability interact with the stress-response, as in one study involving rats, CRH levels increased following the consumption of sucrose but decreased following the consumption of a sucrose/lard mixture (Foster et al., 2009). Further investigations of stress-induced consumption of foods with hedonic properties have relied less on investigations of emotional eating and focussed on theories of addictive eating (Adam & Epel, 2007).

Reward based model of stress eating. Researchers have proposed that increased consumption of palatable foods during stress and the reduction of stress that follows, may be due to the relationship between the stress, feeding, and reward systems (Adam & Epel, 2007). Like drugs of dependence, palatable foods activate dopaminergic, opioid, and endocannabinoid systems, resulting in strong behavioural reinforcement for the consumption of these foods (Adam & Epel, 2007; Cota et al., 2006). Following their consumption during stress, negative feedback of opioid release inhibits the release of CRH and decreases HPA activation, directly inhibiting the stress response in addition to reinforcing consumption of the food (Drolet et al., 2001). "Food addiction," has been explored in terms of sugar consumption resulting in the release of opioids and dopamine, inferring its addictive potential (Avena, Rada, & Hoebel, 2008).

Animal research. Support for the interaction of the reward, feeding, and stress systems has been offered by rat research. The dopamine system was found to be critically involved with increased eating and related behaviours in response to a tail-pinch stressor (Antelman & Szechtman, 1975) and corticosteroids are known to affect dopaminergic reward pathways and

increase palatable feeding in rats (Dallman et al., 2006). Opioids have been found to be directly involved in palatable feeding, as corticosterone replacement increases the willingness of adrenalectimized rats to consume saccharin, suggesting that increased glucocorticoids may increase preference for palatable foods (Bell et al., 2002; Bhatnagar et al., 2000). Similarly, treatment with opioid antagonists has suppressed consumption of these foods (Lowy, Maikel, & Yim, 1980).

Human research. The reward system has been related to palatable feeding in human studies, most notably through comparisons of highly palatable foods to addictive behaviours though these studies to not specifically address the interaction of the stress response with palatable feeding (Avena et al., 2008; Davis & Carter, 2009; Rogers, 2011). Studies previously attributing increased palatable feeding to emotional regulation (Macht & Mueller, 2007; Tomiyama et al., 2011) can be reconsidered under this theory as these studies reveal a reduction of stress following the consumption of highly palatable food but failed to consider the role of reward pathways in promoting this interaction (Adam & Epel, 2007).

Summary. The hedonic properties of palatable foods activate dopaminergic, opioid, and endocannabinoid systems in the brain, reinforcing the consumption of these foods similarly to the reinforcement of drugs of dependence (Adam & Epel, 2007; Cota et al., 2006). This hypothesis cannot however account for all instances of increased eating in response to stress. Wyvell & Berridge (2000) found that changes in dopamine levels may increase the amount of effort rats will exert to obtain palatable food, without changing the palatability of the food. They concluded that dopamine levels in the nucleus accumbens mediated a change in motivation for conditioned responses to receive food (frequency of pressing a lever), without changing the hedonic properties of the food reward (Wyvell & Berridge, 2000). In another criticism of this

theory, fMRI has revealed that despite the choice of more palatable foods under stress, reward signalling was reduced during a stress condition as evidenced by decreased brain activation in reward areas including the amygdala and hippocampus (Born et al., 2010). Despite several proposed explanations for increased feeding in response to stress, it is clear that there must be multiple mechanisms at play in determining whether and individual will eat more or less in response to stress.

The Learning History Model

The proposed model suggests that differences in past learned associations between stress and eating may be a cause for divergent feeding responses in the population. Learning models such as classical conditioning could potentially explain these differences as, for example, if an individual's past exposures to stressful stimuli were followed repeatedly by episodes of overeating, cues associated with stressful conditions may become associated with physiological responses preparing the individual to receive food.

Furthermore, an operant learning model would suggest that increased feeding in response to stress may be a consequence of past negative reinforcement, where increased eating has been repeatedly associated with a reduction of stress or aversive consequences. Therefore, existing learning models can be applied to suggest that overeating and undereating in response to stress may be causally related to differences in past, learned associations made between stress and food intake. However, the influence of past learning on future eating responses to stress represents a gap in the literature and an experimental investigation of the influence of past learning on future eating response to stress is purpose of the present study.

Operant Learning

Operant conditioning involves a response (behaviour), a stimulus (a positive reinforcer or a negative punisher), and a contingency between the response and stimulus (Skinner, 1938). Learning through this model involves either increasing or decreasing the rate of the response of a behaviour through presentation or removal of a reinforcer or a punisher, respectively. Skinner described four response-stimulus (R-S) contingencies that characterize operant learning and the results of these contingencies can be categorized as either pleasant or unpleasant for the subject, promoting an increase or decrease in the rate of response of the behaviour (Skinner, 1938).

Operant conditioning is divided into four categories: positive reinforcement; negative reinforcement punishment training; and omission training, each describing a different response-stimulus contingency (Skinner, 1938). Negative reinforcement and punishment training are particularly relevant to the present study as they involve the use of punishers and can therefore be used to reinforce behaviour contingent on the presentation or removal of a laboratory controlled stressor (Skinner, 1938). Negative reinforcement increases the rate of a response through the removal of a punisher following the desired response, while alternatively, punishment training decreases the rate of a response through the presentation of a punisher following an undesired behaviour (Skinner, 1938). Since the present study seeks to explore eating in response to the presentation of stressful stimuli (which can be considered punishers), these contingencies will be used to mimic the directions of learned overeating and undereating, respectively.

Both classical and operant learning models have been previously used to alter feeding and stress response behaviours in animals. This is evident from the earliest experiments employing these learning models; Pavlov famously demonstrated classical conditioning of

salivation in dogs to the association of a bell (Pavlov & Anrep, 2003) and operant conditioning was used by Skinner to demonstrate that rats could learn to press a lever in order to receive food following repeated reinforcement of food presentation following the lever press (Skinner, 1938). This type of operant responding has been replicated in numerous studies and pressing a lever for intraoral administration of solutions varying in concentrations of high fructose corn syrup was recently conditioned in rats to provide a model for assessing the addictive/reinforcing properties of palatable foods (Levy et al., 2014). The previously discussed reward based model of stress eating further notes the potential relevance of operant learning in understanding increased palatable feeding, as this behaviour has been compared to drug addiction, widely understood to be promoted through positive reinforcement of the rewarding properties of drugs/palatable foods (Weiss, 2005).

The Present Study

The present study seeks to explore the influence of past learning on future eating responses to stress through the application of an operant learning model. To offer support for the proposed learning history model of divergent eating in response to stress, it must first be demonstrated that differences in past learned associations between stress and eating may be sufficient in creating populations with opposite feeding responses to stress. It must further be demonstrated that past associations between stress and eating can generalize to various types of stress, as populations of stress eaters and non-eaters respond consistently in their pattern of eating behaviour to stressors even when exposed to stressors they have not previously experienced and therefore could not have yet associated directly with a change in eating behaviour.

Rats were assigned to a Conditioned Eaters (CE), Conditioned Non-Eaters (CNE), or a Control group, and received respective operant learning that enforced a stimulus-response contingency between eating and the presentation or removal of a specific stressful stimulus (a noise stimulus, procedures that are discussed in more detail to follow). Following operant conditioning, groups were subjected to a test involving presentation of the noise stimulus to determine whether training was effective in producing groups with divergent eating responses to future presentations of this stressor. Further testing sought to explore whether learned associations between increased or decreased eating in response to the noise would generalize to other stressors; in other words, it advised the question, "do divergent eating patterns exhibited by groups when exposed to the noise stimulus reflect a strict stimulus-response contingency between eating and the stressor specifically used in training, or has an association been made between eating and underlying physiological mechanisms activated by the noise stressor as well as by other types of stress?"

Evidence that conditioned eating behaviours can generalize to novel types of stress would support the learning history model of divergent eating responses to stress, offering operant learning as a possible mechanism capable of promoting increased or decreased eating in response to future stress.

Methods

Subjects

Thirty 30-day-old, male Wistar albino rats (*rattus norvegicus),* weighing 250-290 g at the beginning of the experimental period were used as subjects in the present study. Rats were obtained from Charles River Laboratories in Montréal and were housed individually in plastic cages at the Laurentian University Animal Care Facility for the duration of the experiment. Rats were maintained under standard colony conditions on a 12-hour light/dark cycle and were allowed *ad libitum* access to food and water. For each testing session, rats were transported individually, approximately 20m away to an experimental room, weighed prior to testing, and returned individually to their home cages upon completion of testing. All testing sessions took place between 0800h and 1800h. Protocols were approved by the Laurentian University Animal Care Facility.

Materials

Testing equipment. The present study employed a standard operant conditioning box (30 cm x 20 cm x 25 cm) equipped with a pellet dispenser, a stimulus light, and an electrified metal grid floor. Experimental contingencies were controlled by a computer interface program designed by Stanley Koren of Laurentian University. The program allowed for keyboard control over the release of pellets as well as over the on/off times for the noise and light stimuli used in training and testing. Shock was controlled manually with a button on the side of the chamber and the experimenter's movements to control its release were blocked from the view of subjects. Behavioural observations were also recorded by pressing keys assigned to behaviours of interest. The program recorded a timeline of keystrokes, summarizing their frequencies, and saving this information as a .txt file. The experimenter recorded this information, additionally recording

eating latency (latency to begin engaging in eating).. A manual stopwatch was used for timing purposes.

Food. Sugar pellets were chosen to provide a desirable reinforcer for testing phases, as well as to allow subjects to differentiate it from the regular rat chow offered in their home cages. Dustless Precision Pellets (45 mg sugar) were obtained from Bio-serve Inc. as they are a desirable reinforcer and rats can consume a large number of these pellets during a short period of time. These pellets have a caloric value of 3.58kcal/mg and a nutritional profile demonstrating 0% protein, 0% fat, 3.8% fiber, <10% moisture, 0% ash, and 89.5% carbohydrates. These pellets were also selected with consideration to the tendency of humans and animals to choose highly palatable foods when exhibiting increased eating in response to stress (Tomiyama *et al.*, 2012).

Stressors. The stressor used to condition subjects was a loud fragmented tone measured at 98 dB looping on and off at a rate of 100ms/500ms. Noise at this intensity was chosen as the training stimulus because both acute (short-term) and chronic (long-term) noise has been found to elicit stress at this intensity but is not considered harmful to the subjects (Krebs *et al,* 1996). The additional stressors chosen for further testing were a light-stimulus previously associated with shock, a restraint, and a tail-pinch. Foot-shock has been used in many experimental examples to elicit stress as previously discussed, and fear association with unconditioned stimuli and stressful stimuli has been further successful in eliciting the experience of stress (Feenstra, 2000; Mineka & Oehlberg, 2008, Shors, Weiss, & Thompson 1992). Restraint and tail pinching have also both been used frequently as stressors in animal research examining effects on eating behaviour (Greeno & Wing, 1994). The stressor stimuli chosen allowed for all testing to occur within the environment of the operant chamber and allowed for investigation of the influence of stressors of varying qualities and intensities on the behaviours of interest.

Procedure

Food Deprivation. Prior to all testing trials where food consumption was recorded, mild food deprivation schedules were followed where food was removed from the home cages overnight, allowing approximately 18 hours of deprivation, while subjects were allowed *ad libitum* access to water. Testing occurred on six days each week and three groups were always tested on separate days, so rats had at least two days of regular feeding between training and testing days. Rats were not food deprived prior to fear conditioning trials during which food consumption was not involved.

Habituation training. Two weeks after arrival at the colony, rats were subjected to habituation training over 21 days, with each subject completing a total of seven habituation trials. This took place to allow the rats to familiarize themselves with the environment of the operant chamber as well as to introduce and familiarize them to the sugar pellets and provide a baseline measure of pellet consumption following food deprivation. Following food deprivation, rats were transported individually to the experiment room where they were immediately weighed and placed in the operant chamber for one minute of orientation to their surroundings. Five sugar pellets were then released from the pellet dispenser and rats were allowed *ad libitum* access to these pellets with one released by the experimenter each time one was consumed, for 20 minutes. Total number of pellets consumed was recorded, and frequencies of observed urination (number of times urinating), defecation (number of boluses), freezing (>10sec), and excessive grooming (>10sec), as well as eating latency were all recorded by keystrokes and summarized by the experimenter. After 20 minutes, rats were returned to their home cages and were once again allowed *ad libitum* access to regular food and water until their next deprivation.

Group assignment. Following seven habituation trials, each subject's mean eating rate for the final four trials was computed in order to determine their assignment to one of three training groups. Subjects were ordered by increasing pellet consumption and subjects with the closest means were grouped by three and each was then randomly assigned to either a Conditioned Eaters (CE) group, a Conditioned Non-Eaters (CNE) group, or to a Control group. The purpose of this method of assignment was to ensure randomization and equality between groups based on eating rate prior to conditioning.

Conditioning trials. Nine conditioning trials were conducted over 4.5 weeks following the habituation phase, with each group receiving training twice each week. This timeline was designed to ensure that training could occur at approximately the same time of day for all groups and so that subjects would have two days of regular feeding between each food deprivation trial to minimize the stress experienced outside of testing. As with the habituation phase, rats were food deprived 18 hours prior to these trials and were transported to the experimental room individually where they were weighed and placed in the operant chamber. After one minute had passed, to give the rats time to orient themselves, five sugar pellets were released. All subjects had *ad libitum* access to pellets for the remainder of each 20 minute trial. During these trials, different experimental contingencies were applied to each group using the noise stimulus as the punisher for this training. Once again, for all trials, total pellets consumed, as well frequency of urination, defecation, freezing, excessive grooming, and eating latency were all recorded.

The purpose of training trials was to employ an operant learning model to train groups to change their eating behaviour in response to a stressful stimulus, by associating increased or decreased eating with either the presentation or removal of a stressor (in this case the noise). The expected results of training is that when later presented with the noise, the CE group would

increase their eating compared to Control subjects due to learned, repeated associations between increased eating and the removal of the noise stressor in training, while the CNE group would comparatively decrease their eating behaviour due to repeated associations between decreased eating and the removal of the noise stress. The CE group received negative reinforcement training while the CNE group received punishment training, and the Control group received no operant training (procedures further described below).

Negative reinforcement training: Conditioned eating (CE). For the CE group, a schedule of negative reinforcement training was followed and the tone was presented immediately following one-minute of orientation to their surroundings. The tone was turned on at the beginning of each trial and remained on, being turned off when the subject began to eat. After the subject stopped eating for 30 seconds, the tone was presented again, and these contingencies lasted for the duration of each 20-minute trial. Therefore, as long as the CE rats continued to eat, the noise remained off. Subjects were exposed to the stressor whenever they stopped eating, with the intention being for rats to learn to associate increased eating with the removal of a stressor.

Punishment training: Conditioned non-eating (CNE). For the CNE group, a schedule of punishment training was followed. Following one-minute of orientation, the tone was left off and was turned on only when the subject began to eat. The tone was removed after 30 seconds of no eating and was presented again whenever the subject resumed eating. These contingencies again lasted for the duration of each 20-minute trial. Therefore, as long as the CNE rats did not eat, the noise remained off, exposing subjects to the stressor only when eating, with the intention being for rats to learn to associate decreased eating with the removal of a stressor.

Control group procedures. For the Control group, all other testing contingencies were followed and behaviours were recorded, however no stressor was presented by the experimenter during these trials. Prior to the noise test, the Control group therefore had no exposure to the noise stressor so that they would make no direct association between eating and any experimental stimulus. For the Control group, conditioning trials therefore followed identical procedures as the habituation trials.

Testing phases. Following habituation and training were five testing phases, with three additional training trials conducted following each test involving the presentation of a novel stressor (light, restraint, and pinch). These additional trials followed the same procedures as the original training received by each group. For all testing, procedures remained the same within the CE, CNE, and Control groups. Rats were again transported to the experimental room individually following food deprivation, they were then weighed and placed in the operant chamber for one minute of orientation. Pellet consumption as well frequency of urination, defecation, freezing, excessive grooming, and eating latency were all again all recorded.

Test 1 – Noise test. The purpose of the noise test was to verify whether or not operant conditioning was effective in causing groups to eat different amounts when exposed continuously to the noise stimulus used in training. During this test, orientation was followed by a 20-minute period during which the aversive tone (used in conditioning trials) was turned on for the entire duration of testing and rats were given *ad libitum* access to sugar pellets. If the CE group learned, from their history of negative reinforcement training, that eating led to the removal of the stressful noise, we should expect that they would significantly increase their food intake compared to the Control group when presented with this stimulus. Furthermore, if the CNE group learned, from their history of punishment training, that eating led to presentation of

the noise, we would expect that they would significantly decrease their pellet consumption compared to the Control group during this test.

Test 2 – No-stress test. The purpose of the no-stress test was to verify that significant differences in eating behaviour between groups were not present in the absence of stress, supporting that divergent group eating responses observed in other tests were a result of exposure to the experimental stressors being presented, and not a result of exposure to other stimuli present in the testing environment during both testing and training procedures. This time, orientation was followed by a 20-minute period during which the noise stressor used to condition subjects remained off (for the entire duration of testing), while rats were given *ad libitum* access to sugar pellets.

Test 3 – Light test. The purpose of the following tests (Tests 3-5) was to examine whether or not changes to group eating behaviour observed during the noise test would generalize to exposure to other types of stressors. If obtained, significant differences in eating behaviour between groups during light, restraint, and tail-pinch tests, this would suggest that the effects of conditioning on group eating behaviour during the noise test were not caused by the presence of the noise-stressor specifically, but instead may have been the result of a generalizable association formed between eating behaviour and internal stress-cues elicited similarly by the noise-stressor and by other types of stress-inducing stimuli. In other words, these experiments sought to explore whether the learning history gained during training would generalize to other stressors, affecting the eating response of subjects when exposed to stressors they had no prior exposure to.

The light test involved two stages: i) light/shock association (with each group receiving two association trials) and ii) a testing day for each group during which the light stimulus was

present for the duration of the test and subjects were not exposed to shock. By pairing a light stimulus (conditioned stimulus) and electric foot-shock (unconditioned stimulus), rats were expected to develop a conditioned fear (conditioned response) to the light due to its association with the shocks. Subsequent presentations of the light stimulus, without shock, were expected to elicit this fear response, producing a stressful experience for subjects. As food consumption was not involved in this stage of experimentation, animals were not food deprived prior to fear conditioning trials. During these trials, orientation was followed by turning on a light stimulus (1inch, round, yellow light) affixed to the wall of the operant chamber that would remain turned on for the duration of each 20minute association trial. During this time, electrical foot-shocks (63 volts and lasting three seconds) were released through the metal grid floor of the operant chamber by the experimenter at 10 predetermined, randomized times. This protocol was followed for both fear-conditioning trials allowing multiple chances for each rat to form a potential association between the light and shock. Behavioural measures unrelated to food consumption were still recorded.

Testing occurred three days after the second fear-conditioning trial; rats were food deprived according to their regular schedules and orientation was followed by a 20-minute period during which the stimulus light was turned on and remained on for the duration of the trial while rats were given *ad libitum* access to sugar pellets and received no shock. Pellet consumption and behavioural measures were once again recorded.

Test 4 – Restraint test. The restraint test presented subjects with yet another form of stress, as they were secured in restraint tubes for 15 minutes prior to being released into the operant chamber where no other aversive stimuli were present during the test. Restraint tubes were 3.5inches in diameter and 9 inches in length, made of white plastic and designed by

specifications for rats weighing 500-800g, suggested by Plas-Labs Inc. As in other tests, rats were given *ad libitum* access to sugar pellets, and pellet consumption and behavioural measures were again recorded.

Test 5 – Pinch test. Finally, the pinch test presented a third form of novel stress to subjects with the addition of a tail pinch. As in previous literature, a padded paperclip was used to pinch rats' tails approximately two centimeters from the tip for the duration of testing for all groups (Stengard, 1994; Pei, Zetterstrom, & Fillenz, 1990; Chang, Liao, Lan, & Shen, 2000). Paperclips were padded with two layers of electrical tape and were affixed with tape after being applied to subjects in order to avoid the clip falling off. This procedure was piloted on a rat not involved in this experiment, revealing the necessity to alter the operant box slightly for this testing phase; a sheet of clear plastic was affixed below the metal grid floor because the paperclip was likely to fall off during testing if it slipped between the bars of the operant chamber. The paperclip was applied immediately prior to placing the rat in the operant chamber for orientation. As in all other tests, pellet consumption and behavioural observations were recorded for the duration of the 20-minute trial.

Follow up tests. 10 days after this first phase of training and testing, follow up tests were performed during which procedures for the noise test and no-stress test were repeated, this time with the no-stress test occurring first for each group to ensure that similar eating by groups observed in the first no-stress test was not promoted by potential extinction effects of the noise test having already occurred. Repeating these tests would further allow for analysis of whether eating behaviour during follow up tests differed from initial testing, perhaps because of subject weight gain or due to continued increased familiarization with the testing environment and stimuli presented, over time. All procedures remained the same as for the noise test and the no-

stress test, but with the follow up no-stress test occurring in the days prior to the follow up noise test.

Results

Habituation Analysis

To verify that groups did not significantly differ in baseline pellet consumption prior to conditioning, a one-way analysis of variance (ANOVA) was conducted to verify that all three groups ate similar amounts based on the mean eating behaviour values used for group assignment. Mean pellet consumption for the final four habituation trials (used as grouping averages) were therefore compared by group and no significant differences were noted, $F(2,27)=.014$, $p=.986$. To further ensure that group differences in eating behaviour did not exist between groups prior to conditioning, a repeated-measures ANOVA was conducted, comparing eating during all habituation trials (H1-7), by retrospective group assignment. There was a main effect of trial ($F=(2.88, 77.66)=48.423$, $p<.001$, $\eta p^2=.642$) but neither a main effect of group nor a group by trial interaction were noted. For the main effect of trial, contrasts revealed a significant linear relationship ($F(1,27)$-211.083, $p<.001$, $\eta p^2=.887$). It is clear from a graph comparing group eating by habituation trials (Figure 1), that group differences were not present during any trial and that overall, groups increased eating as the habituation phase progressed. This main effect was an expected result of increased maturation of subjects and their continued familiarization with the sugar pellets and with the testing environment.

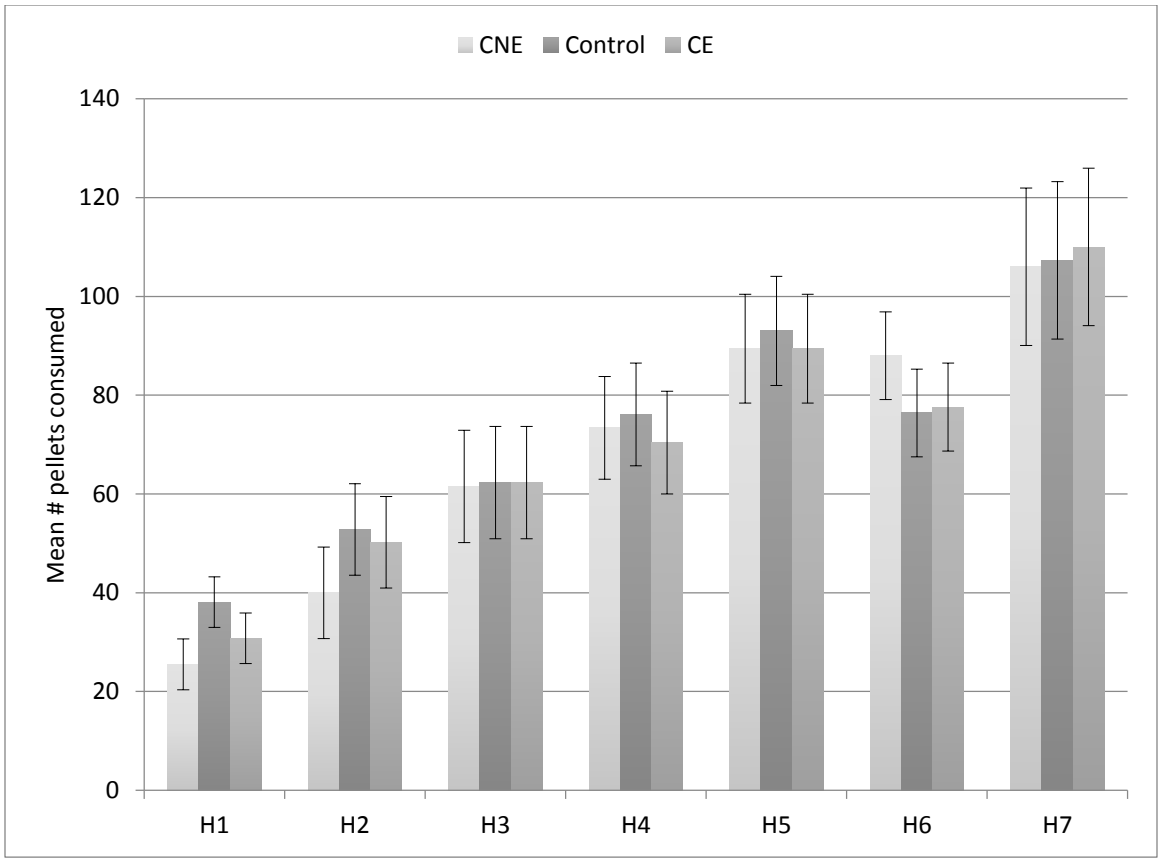

Figure 1. Mean eating behaviour during habituation trials by groups. Error bars indicate SEM.

Conditioning Analysis

To examine how eating behaviour was affected as training progressed, a repeated-measures ANOVA was conducted comparing group eating behaviour for the nine conditioning trials (C1-9). All effects were significant, with a main effect of group ($F(2,27)=8.079$, $p=.002$, $\eta p2=.374$), a main effect of trial ($F(4.87, 131.42)=13.853$, $p<.001$, $\eta p2=.339$), and a group by trial interaction ($F(9.74, 131.42)=6.540$, $p<.001$, $\eta p2=.362$) reported.

The main effect of trial revealed significant differences in eating between trials, demonstrating that eating rate during later trials was significantly increased compared to earlier trials. Most notably, corrected multiple comparisons revealed that subjects ate less during trial

C1 than trials C3-C5 ($p<.05$) and C7-C9 ($p<.001$) and subjects ate significantly more during trials C7-C9 than during trials C2 and C5 (both $p<.05$). Post-hoc comparisons by group revealed that the CNE group ate less overall than the CE group ($p=.001$) as well as the control group ($p<.05$), however a significant effect of trial was not reported between the control and CE groups.

Multiple comparisons of the group-by-trial interaction revealed no significant group differences for early trials (C1-C3), though as early as trial C4, group differences were significant and this pattern of eating behaviour persisted for trials C5-C9. The CE and CNE groups demonstrated divergent eating behaviour as the CNE group reduced their pellet consumption compared to the CE and Control groups, and the CE group increased their eating significantly compared to the Control group as well. These trends are illustrated in Figure 2 and effects are summarized in Table 1.

Table 1

Summary of group effects by conditioning trials

*(*denotes significance at p<.05; **denotes significance at p<.001)*

Trial	*F*(2,27)	*p*-value	ηp2 of significant effects	Significant effects observed:
C1	1.641	.212	--	--
C2	2.612	.092	--	--
C3	2.492	.102	--	--
C4	4.037	.029*	.230	- CNE < CE
C5	5.597	.009*	.293	- CNE < CE - CNE < Control
C6	4.957	.015*	.269	- CNE < CE
C7	10.354	<.001**	.434	- CNE < CE - CNE < Control

C8	20.794	<.001**	.606	- CNE < CE - Control < CE
C9	17.902	<.001**	.570	- CNE < CE - CNE < Control - Control < CE

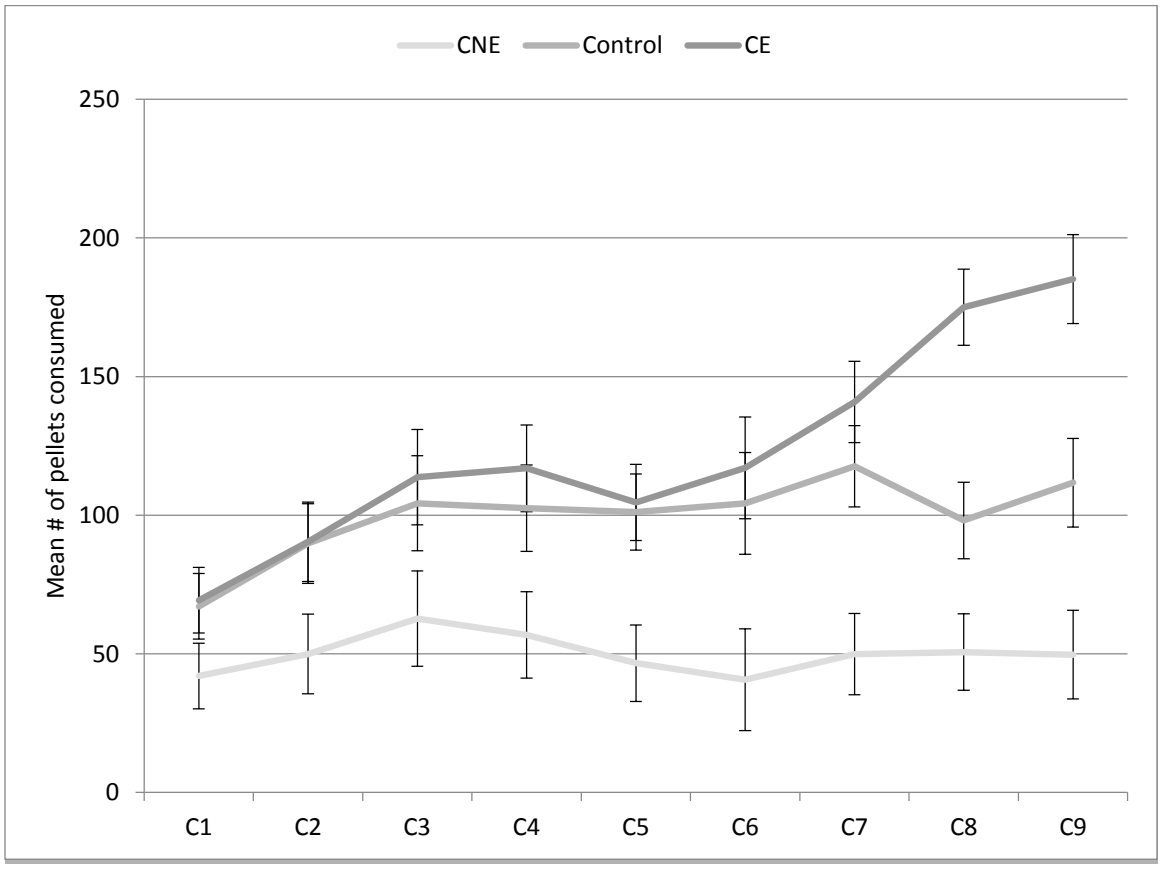

Figure 2. Mean eating behaviour by group for all conditioning trials. Error bars indicate SEM.

Examining comparisons by-group further revealed no significant effect of trial for the CNE group but did reveal significant effects for the CE ($F(8,20)=17.098$, $p<.001$, $\eta p2=.872$) and Control groups ($F(8,20)=2.492$, $p<.05$, $\eta p2=.499$). The CE group ate more during trials C8 and C9 than during all other trials ($p<.05$) and further ate less during trial C1 than trials C3-C5 ($p<.05$) and C7-C9 ($p<.001$). This pattern likely drove the previously reported main effect of

trial. The Control group further ate less during the first training trial (C1), than during trials C5, C7, and C9 ($p<.05$).

Results indicate that as conditioning progressed, the eating behaviour of the CE and CNE groups differed from the Control group. Compared to the Control group, the CE group increased eating over time, while the CNE group demonstrated reduced eating compared to the Control group. For the CNE group, differences in eating compared to the Control group emerged earlier than for the CE group, possibly accounting for why significant differences in eating behaviour were not observed across trials within this group. Further, increased eating behaviour by the Control group across trials appeared to plateau by C4 when significant group differences were first reported. This could be explained by subjects eating more as they matured or because of continued familiarization with the testing environment. Results suggest that training methods were effective in producing group differences in eating behaviour, as the CE group learned to increase while the CNE group learned to reduce their eating compared to the Control group as a result of the repeated associations made between eating and contingent presentation and removal of the noise stressor during these trials.

Evaluating the Effectiveness of Conditioning

Before examining the effects of multiple stressor-types on group eating behaviour, pellet consumption during the noise and no-stress tests were compared by group for both initial and follow-up tests. The purpose of this comparison was to determine whether operant conditioning had successfully caused the CE group to eat more and the CNE group to eat less than the Control group, when presented with the noise stimulus used in training. Furthermore, it was important to verify that group differences caused by training did not occur during the baseline eating behaviour of groups when no stressor was present but all other conditions remained the same. If

differences in eating between groups were still present in the absence of a stressor, it would indicate that differences in pellet consumption observed during exposure to stress were due to an overall change in eating behaviour that was not specifically elicited by the presence of the stressor.

A repeated-measures ANOVA compared group by stressor-type (noise or no-stress) by time (initial testing or follow up testing). There were significant main effects for stressor-type ($F(1,27)=9.794$, $p=.004$, $\eta p2=.756$) and group ($F(2,27)=11.072$, $p<.001$, $\eta p2=.451$), and a significant group-by-time ($F(2,27)=4.203$, $p<.05$, $\eta p2=.483$) and group-by-stressor ($F(2,27)=41.909$, $p<.001$, $\eta p2=.756$) interactions were reported as well. There was neither a main effect of time nor interactions of time by stressor or time by stressor by group.

Post-hoc tests revealed that overall eating was reduced for the CNE group compared to the CE group ($p=.001$) and the control group ($p=.007$) but overall differences between the CE and control groups were not significant. The main effect of stressor-type revealed that all subjects significantly reduced eating when the noise stressor was present compared to when no stressor was present ($p=.004$). Corrected multiple comparisons of the time by group interaction revealed that groups ate significantly different amounts during both initial ($F(2,27)=12.607$, $p<.001$, $\eta p2=.483$), and follow up ($F(2,27)=8.781$, $p=.001$, $\eta p2=.394$), tests. During initial tests, the CNE group ate significantly less than the CE group ($p<.001$) and the control group ($p<.05$), and these significant trends persisted during follow up tests (at the same levels of significance). The time-by-group interaction was further significant only for the CNE group ($F(1,27)=4.729$, $p<.05$, $\eta p2=.149$), eating more during follow up tests than during initial testing. Important to note, is that this interaction does not account for differences in stressor-type and that group differences were likely driven by significant differences during the initial and follow up noise

tests. The absence of a main effect for time, indicates that overall eating behaviour was not affected by time between tests nor by intermediate testing and training that took place. The significant effect of time for the CNE group (increased eating during the follow-up tests) could possibly reflect a decreased experience of stress due to continued familiarization with the noise stressor during intermediate training and testing.

The group-by-stressor interaction was significant only for the noise tests ($F(2,27)=30.506$, $p<.001$, $\eta p2=.693$), and group differences did not persist during the no-stress tests. During the noise tests, the CNE group ate less than the CE group ($p<.001$) as well as the Control group ($p=.001$), and the CE group ate more than the Control group ($p=.004$). These results support our prediction that operant training paradigms were effective in causing divergent group eating behaviour in response to exposure to the noise stimulus used to train them. As well, these results verify that changes in eating behaviour were dependent on exposure to a stressor, since group differences were not observed in the absence of stress. The group-by-stressor interaction further revealed significant differences in eating between groups, as when tested with the noise stressor compared to no-stress, the CE group increased eating ($F(1,27)=23.927$, $p<.001$, $\eta p2=.407$), the CNE group decreased eating ($F(1,27)=64.478$, $p<.001$, $\eta p2=.705$), the and the Control group also decreased their eating behaviour ($F(1,27)=5.209$, $p<.05$, $\eta p2=162$). These trends are illustrated in Figure 3.

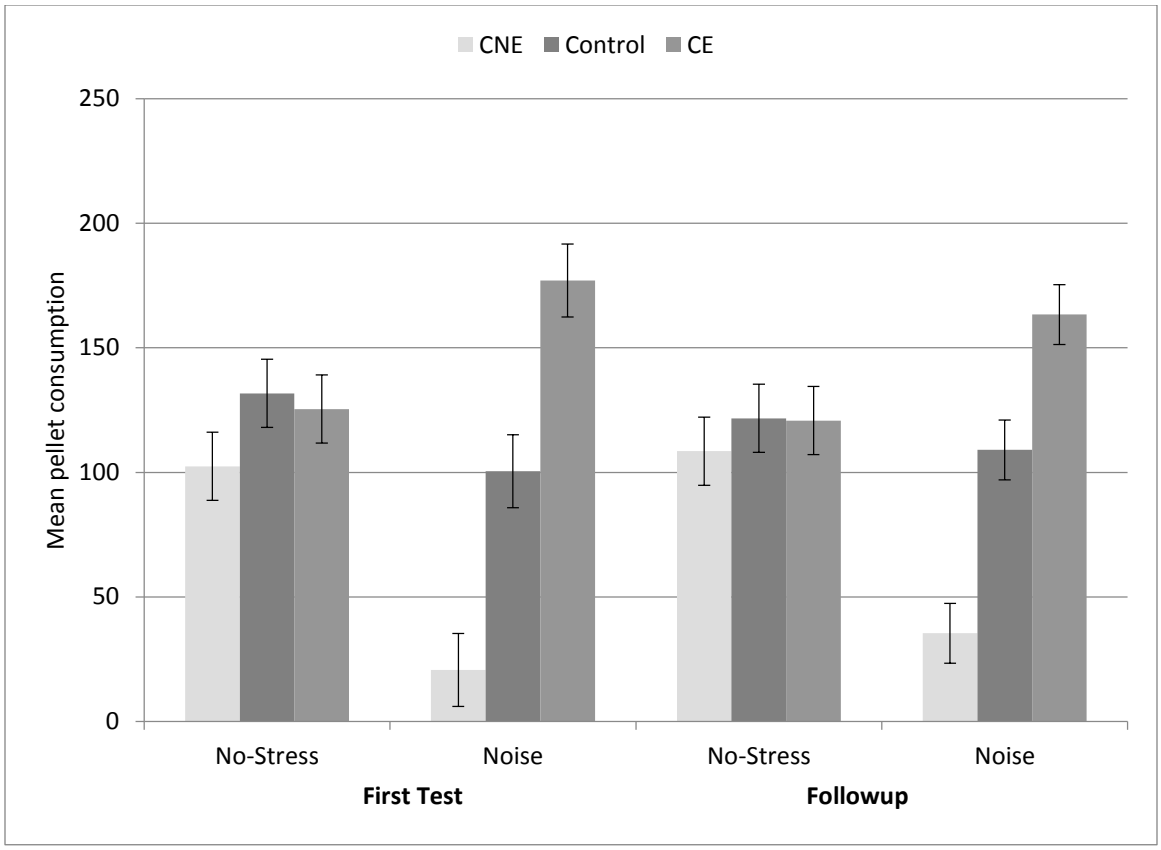

Figure 3. Mean eating behaviour by group for no-stress, noise, and follow-up tests. Error bars indicate SEM.

Test Analysis

To examine the effects of the light, restraint, and pinch stressors on group eating behaviour, a repeated-measures ANOVA was conducted comparing pellet consumption by group for all stressor-types (no-stress, noise, light, restraint, and pinch). All effects were significant, with main effects reported for group ($F(2,23)=19.288$, $p<.001$, $\eta p2=.626$), and stressor-type ($F(2,23)=16.828$, $p<.001$, $\eta p2=.423$), as well as a group-by-stressor interaction ($F(8,92)=7.00$, $p<.001$, $\eta p2=.378$). For the main effect of group, post-hoc tests revealed that overall, the CNE group reduced eating compared to the CE ($p<.001$) and Control groups ($p=.001$). For the main effect of stressor-type, groups significantly reduced eating behaviour during the light ($p<.001$), restraint ($p=.001$), and pinch ($p=.003$) tests compared to during the no-stress test and

furthermore, groups ate less during the light test than the noise (p=.002), restraint (p=.009), and pinch tests (p=.003).

The group-by-stressor interaction revealed that groups ate significantly different amounts during the noise (F(2,23)=22.909, p<.001, ηp2=.666), light (F(2,23)=23.154, p<.001, ηp2=.668), restraint (F(2,23)=5.431, p<.001, ηp2=.321), and pinch tests (F(2,23)=30.689, p<.001, ηp2=.727), but ate similar amounts during the no-stress test. Corrected multiple comparisons revealed that within this interaction, the CNE group ate less than the CE (p<.001), and Control groups (p=.004), while the CE group ate more than the Control group during the noise, light, and pinch tests (all comparisons significant at p<.05). For the restraint test, the CNE group ate significantly less than the CE and Control groups (both p<.05) but significant differences were not observed between the CE and Control groups. These results infer that groups responded with similar patterns of behaviour during tests involving the presentation of new stressors, while displaying similar eating patterns when no stressor was present.

The group-by-stressor interaction further revealed eating behaviour differed between stressor-types for the CE (F(4,20)=6.894, p=.001, ηp2=.570), CNE (F(4,20)=14.259, p<.001, ηp2=.740), and Control (F(4,20)=6.139, p=.002, ηp2=.551) groups. Compared to the no-stress test, the CNE group reduced their eating behaviour during the noise (p<.001), light(p<.001), restraint (p=.004), and pinch (p=.008), tests and further ate less during the light test than the restraint or pinch tests (both p<.05). The CE group increased eating during the noise test compared to the no-stress test (p<.001) and further ate more during the noise test than during the light(p=.005), restraint (p<.001), and pinch tests (p=.005). Finally, the control group ate significantly less during the light test than during the no-stress (p<.001), and restraint (p=.014) tests. These trends are illustrated in Figure 4.

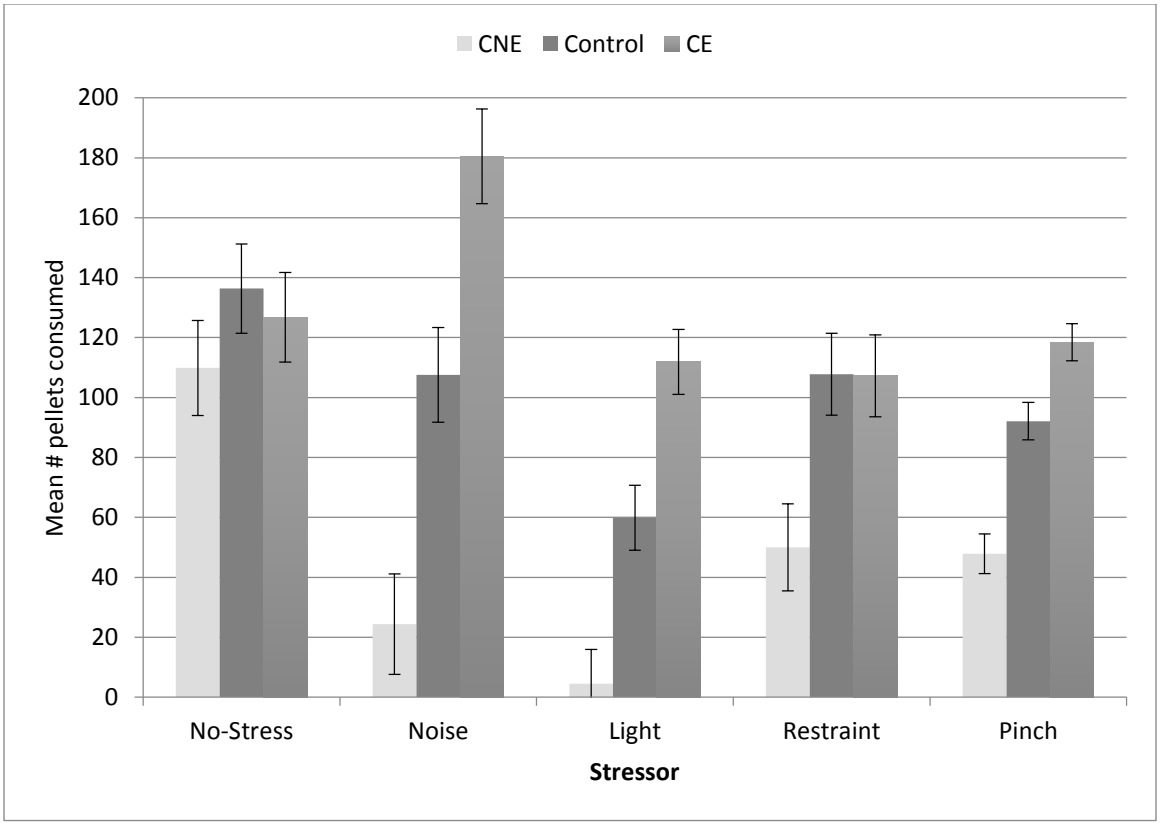

Figure 4. Mean eating behaviour by group for all stressor-types.
Error bars indicate SEM.

Behavioural Analysis

Finally, a repeated-measures ANOVA compared behavioural measures for all groups and each test, in order to investigate how these behaviours were influenced by stressor-type. Five measures were included in the analysis; urination, defecation, freezing frequency, grooming, and eating latency were included. Analysis of the Control group's behaviours within this interaction were of particular interest as previous literature has investigated the effects of stress on these behaviours in normal populations with mixed results. A significant effect of test was reported for all variables, and a main effect of group, as well as a group-by-stressor interaction reported for freezing and eating latency. These effects are summarized in Table 2.

Table 2

Results and significant effects of repeated measures ANOVA comparing behavioural measures by stressor-type and group.

*(*denotes significance at p<.05; **denotes significance at p<.001)*

	Results	Significant Effects
Main Effect: Stressor-type		
Urination	$F(4,108)= 2.723$, $p<.05$, ηp2=.092	--
Defecation	$F(2.6, 69.4)= 18.121$, $p<.001$, ηp2=.402	No-stress < Light** < Restraint** Noise < Light* < Restraint* Pinch < Restraint*
Freezing	$F(2.3, 62.1)= 38.272$, $p<.001$, ηp2-.586	No-stress < ALL* ALL < Light**
Grooming	$F(4,108)=14.747$, $p<.001$, ηp2=.353	No-stress > Light** > Pinch* Noise > Light* Restraint > Light** > Pinch*
Latency	$F(2.7,72.2)= 4.134$, $p<.05$, ηp2=.133	No-stress < Restraint*
Main Effect: Group		
Freezing	$F(2,27)= 8.483$, $p=.001$, ηp2=.386	CE < CNE**
Latency	$F(2,27)= 9.018$, $p=.001$, ηp2=.400	CE < CNE*
Group x Stressor-type Interaction		
Freezing	$F(4.6,62.1)= 3.570$, $p<.05$, ηp2=.209	**CNE** No-stress < Noise* ALL < Light** **Control** No-Stress < Noise* < Light* Restraint < Light* Pinch < Light*
Light:	$F(2,27)= 6.678$, $p<.05$, ηp2=.331	CE < CNE*
Latency	$F(5.4,72.2)=2.824$, $p<.05$, ηp2=.173	**CNE** ALL < Restraint*
Restraint:	$F(2,27)= 8.154$, $p<.05$, ηp2=.377	CE < CNE* Control < CNE*

While significant results do not appear to represent consistent patterns of responding between behavioural measures of interest, freezing and eating latency demonstrated interactions between group and stressor-type worth noting. Freezing was of particular interest as main effects and interactions observed for this behaviour supported the hypothesis that tests involving the presentation of a stressor resulted in increased freezing overall compared to when no stressor was present, with freezing frequency further increased during the light test compared to all other tests. The main effect of group on freezing behaviour indicated that the CNE group froze more than the CE group and the interaction further indicated that these main effects were likely driven by effects of the light test and by behaviour of the CNE group.

Increased freezing by the CNE group occurred during tests during which this group demonstrated decreased eating, supporting previous literature proposing that increased freezing behaviours are likely correlated with increased stress. Consistent with this, increased freezing was also observed in the Control group as they decreased eating during the light and noise tests compared to the no-stress test. Increased freezing and eating latency by the CNE group compared to the CE group, could perhaps reflect that stress-induced behaviours were observed less for the CE group due to more time being spent engaged in eating behaviours, or to an inhibition of the stress response experienced by this group due to increased consumption of sugar resulting in a decreased experience of stress. These results will be further discussed in relation to current literature in the following section. Not surprisingly, grooming demonstrated a pattern of results opposite to other behavioural responses, appearing to decrease in frequency during tests involving exposure to stress, suggesting that grooming may be more likely reduced during stress, though this pattern not consistently reported for all tests. Finally, the main effect of defecation

was generally consistent with increased defecation occurring during increased stress, significantly in light and restraint tests compared to the no-stress test as well as the noise test.

Discussion

As discussed, previous research has yielded conflicting results with regard to the effects of stress on eating. While the current study presents findings that are consistent with the literature with regard to the notion that stress may cause some individuals to increase and others to decrease eating, what the present literature lacks is investigation into which individual factors may contribute to these divergent responses (Greeno & Wing, 1994). The present study was designed to investigate whether differences in past learning may play a significant role in determining whether individuals will eat more or less during stress. The influence of different operant learning histories on eating behaviour exhibited by rats exposed to stress was examined by employing two operant training paradigms: negative reinforcement and punishment training. A noise stressor was used as the punisher in this design and an animal model was used to determine firstly, whether repeated associations between the noise stressor and either increased or decreased eating, would result in groups demonstrating these feeding responses when exposed to the same stressor in future testing.

Results from the noise and no-stress tests indicate that training was effective in causing groups to respond by eating different amounts during later exposure to the noise stressor used for training. During the stress condition, the Conditioned Eaters (CE) group increased their food intake and the Conditioned Non-Eaters (CNE) group decreased their food intake significantly, compared to the Control group. During the no-stress tests, subjects were not exposed to any experimental stressors and all groups ate similar amounts, demonstrating that differences observed between groups during other tests involving stressors, must have been dependent on exposure to the stimuli presented during those tests. These stress-related differences in food intake were also observed when the rats were presented with novel stressors that groups had no

prior exposure to, suggesting that the contingencies of training generalized to future experiences of stress, induced similarly by different types of aversive stimuli. Before delving into the implications of present findings and their contribution to the literature investigating the stress-eating relationship, explanations of eating patterns observed for groups during training and testing will be discussed as they relate to present hypotheses and current literature.

Training. Prior to testing, the training phase functioned to condition groups through the implementation of negative reinforcement and punishment training. The CE group was expected to eat more as training progressed (associating eating with the cessation of the stressor), the CNE group was expected to eat less (associating eating with the commencement of the stressor), and the Control group was expected to eat similar amounts during these trials (no association between eating and the stressor The group by trial interaction indicated that group differences emerged by the fourth trial with the CNE group eating less than the CE group when the stressor was presented. By the fifth trial the CNE ate less than the Control group, and by the eighth trial the CE group ate more than the Control group when the stressor was presented. This interaction further revealed that the CE and Control groups ate more during later trials, while the CNE group demonstrated no change in eating behaviour between early and late trials. Possible explanations for increased feeding by the Control group during these trials, are continued habituation to the food and testing environment, as well as increased maturation of subjects over time. *Figure 4* (p.46) illustrates that pellet consumption by the Control group did appear to plateau following the first trial, the only trial during which this group demonstrated decreased eating compared to other training trials. While the CNE group demonstrated no significant changes in eating from the first to final training trials, a pattern of decreased eating by the CNE group compared to the Control group emerged over training, earlier than differences emerged between the Control and

CE groups. That punishment training was effective sooner than negative reinforcement in this model, suggests that punishment training may have been more effective, though it is important to consider that these results may be influenced by a general effect of noise stress on eating behaviour in the absence of training.

Noise and No-Stress Tests. While it was not the focus of this study, analysis of feeding behaviour by the Control group during tests involving exposure to stressors compared to during the no-stress test, allowed for insight into how the stressors used would affect feeding behaviour in the absence of training. Consistent with reports that stress most often leads to reduced feeding behaviour in rats (Greeno & Wing, 1994) exposure to the noise stressor caused the Control group to eat less than they did in the absence of stress. The noise test represented the Control group's first exposure to the stimulus that other CE and CNE were exposed to in training, therefore decreased feeding by the Control group during this test demonstrates a general effect of decreased eating caused by exposure to the noise stressor in the absence of training. These results imply that exposure to the noise should cause subjects to eat less, and this being congruent with the desired effect of punishment training may account for training having had a sooner impact on the feeding behaviour of the CNE group than the CE group (Figure 4).

While Control group behaviour during the noise and no-stress tests suggests a general effect of reduced eating caused by the noise, these tests were meant to examine the effects of operant conditioning on how groups would respond to the noise following training. Results of the noise test compared to the no-stress test support that training caused the CE group to increase and the CNE to decrease how much they ate when exposed to the noise, compared to the Control group and compared to when no stressor was present. When the noise stress was absent but all other testing conditions remained the consistent, groups ate similar amounts. These findings

suggest that differences in eating behaviour between groups were contingent on the presence of the noise stimulus rather than the environment of testing conditions, and were not the result of a permanent change of behaviour resulting from the experimental manipulations of training. These results were expected, as operant learning is an effective method of producing increased versus decreased responding of various behaviours using negative reinforcement and punishment training, respectively. In the present study, the noise stressor was considered a punisher that was associated with increased or decreased feeding behaviour in training through contingencies of operant learning, with results of the noise and no-stress tests supporting that this desired learning was achieved. The CE group learned to associate increased eating with the removal of the noise stress and responded by eating more, while the CNE group learned to associate decreased eating was associated with exposure to the noise stress and consequently responded by eating less during later exposure to the noise.

Changes to the feeding behaviour of rats in response to stressors similar to those used in this study have been previously researched and the present experiment demonstrates results both consistent and inconsistent with previous literature. Chronic (long term) noise stress has been found to cause decreased feeding in rats (Alario et al., 1987), while acute (short-term) noise has previously resulted in increased feeding (Krebs, Macht, Weyers, Weijers, & Janke, 1996; Krebs, Weyers, Macht, Weijers, & Janke, 1997; Kupfkrmann, 1964). However, in the present experiment, acute noise stress led to decreased feeding by the control group during their first exposure to the noise stressor. A possible explanation for this contradiction to previous studies is that differences in stressor quality and intensity may have influenced results. The stressor used in this experiment was a fragmented tone measuring 96 dB that looped on and off at a rate of 100 ms/500 ms, while previous studies used noise stressors defined as 95 dB white noise (Krebs et

al., 1996, 1997) as well as clicking/whistling sounds measuring 60-80 dB (Kupfkrmann, 1964), and it is further notable that general effect hypotheses have not been supported consistently in past investigations involving various types of stressors (Greeno & Wing, 1994).

While results of the noise and no-stress tests were an expected result of operant learning, differences in eating between groups observed during the noise test that could be reproduced by exposure to other types of stressful stimuli, would suggest that group differences observed in the noise test did not simply reflect learned associations between feeding behaviour and the punishment of the noise stressor specifically, but instead reflected associations having been made between increased or decreased eating and the internal stress response that can be elicited similarly by the noise stimulus and by other types of stressful stimuli. To determine whether trained eating responses to the noise could generalize to other stressors, following tests investigated whether eating behaviour differences between groups observed in during the noise test, would persist when subjects experienced stress caused by novel stressors they had not previously been exposed to.

Light Test. The light test used a light stimulus (CS+) as a stressor that signalled the threat of shock (US) following prior fear-conditioning trials received by all groups. During this test, patterns of behaviour observed during the noise test persisted, as the CE group increased feeding compared to the Control group while the CNE group decreased their feeding response. These effects were larger than for other tests involving novel stimuli, and light was the only stimulus aside from the noise stressor that caused the Control group to reduce their eating compared to when no stressor was present. While the effects of stress on the feeding behaviour of rats have not been previously examined using a light stimulus associated with shock as a stressor specifically, fear conditioning in rats involving context dependent association with a

single session of exposure to shock, has previously increased corticosterone levels in rats (Cordero, Venero, Kruyt, & Sandi, 2003). This is consistent with our behavioural measures suggesting that exposure to the light stimulus was effective in causing subjects to experience stress following fear conditioning (increased freezing during the light test compared to during the no-stress test and to other tests by the CNE and Control groups).

While shock as a stressor has resulted in a range of effects on the feeding behaviour of rats (Siegel & Brantley, 1951; Solomon et al., 2007; Sterritt, 1965; Strongman, 1965; Tugendhat, 1960; Ullman, 1951, 1952), it has been proposed that fear of shock may lead to decreased eating, as in one experiment where rats were exposed to unavoidable shock, rats increased eating while shocked, but reduced eating compared to unstressed controls while in the same environment when the shock was off and subjects were conceivably stressed by the anticipation of being shocked (Sterritt, 1962). This is consistent with present results that the Control group significantly reduced their eating during the light test despite not receiving training involving associations between stress and reduced eating. Fear has also been used as a stressor resulting in decreased feeding by humans; one study showed that normal weight individuals reduced their eating when frightened compared to when calm (Schachter et al., 1968), and in a comparison of restrained and unrestrained eaters, anticipation of electric shock was considered a physical fear threat that resulted in significantly reduced eating by normal eaters (Heatherton et al., 1991). Behavioural results of the present study suggest that increased freezing indicative of stress was influenced more strongly during the light test than by other stressors tested. Results support that the light stimulus associated with shock was effective at causing rats to experience a stress response and that general effect of decreased feeding in response to this stressor was observed by Control subjects in response to this stimulus.

Restraint Test. Restraint stress has resulted in decreased eating by rats fairly consistently in past research (Calvez et al., 2011; Martí et al., 1994; Rybkin et al., 1997), however results from the present study suggest that our Control group did not change their eating behaviour during the restraint test compared to the no-stress test. This was also the only test during which increased feeding was not observed by the CE group compared to the Control group, though the CNE group reduced their eating compared to the Control group. It is possible that similar eating behaviour by the CE and Control groups, as well as similar eating by the Control group during the restraint and no-stress tests, may have been influenced by the timing of stimulus exposure, as subjects were exposed to the restraint stressor for 15 minutes prior to the testing period, while other stressors were presented continuously throughout the 20 minutes when feeding behaviour was observed. While the CNE group reduced eating compared to the Control group, the CNE group additionally had a longer eating latency during the restraint test compared to all other tests. This demonstrates a significant effect of training on feeding behaviour at the beginning of this test, immediately following release from the restraint stress, as subjects in the CNE group took longer to initiate feeding than the Control group.

However, it is also possible that failure of the restraint stress to inhibit eating by the Control group could be explained by the aversive state reduction hypothesis, as restraint stress has been equated to emotional stress and has been found to increase preference for sweet foods like the sugar pellets that were used in this study (Alario, Gamallo, Beato, & Trancho, 1987). While this effect has not consistently been observed for acute restraint stress (Ely et al., 1997), this theory would support an explanation that significant differences between the CE and Control groups may have existed if sweet food had not been used as it is more likely to cause increased feeding in response to an emotional restraint stress specifically.

Tail-Pinch Test. Finally, while mild tail-pinches have had the general effect of causing increased feeding in several studies (Antelman & Szechtman, 1975; Dess, 1997; Levine & Morley, 1981, 1982; Rowland & Antelman, 1976), present results of Control group behaviour during this test were consistent with studies demonstrating no effect of tail-pinch stress on the feeding behaviour of rats (Meadows et al., 1988) as the Control group's eating was not affected compared to when no stressor was present. Group differences in feeding behaviour were again reported for this test, following the pattern of behaviour observed by groups during the noise test, as the CNE group demonstrated reduced feeding compared to the Control group and the CNE group increased feeding compared to the Control group.

Implications

Results for the restraint, light, and tail-pinch tests demonstrate that increased eating by the CE group and decreased eating by the CNE group observed during the noise test, persisted during exposure to novel stressors as well, suggesting that operant learning involving associations with one type of stressor can affect the eating behaviour of rats when exposed to other types of stressful stimuli as well. Therefore, associations that were made between changes in feeding behaviour in response to the noise stressor, were extended to other stimuli activating a similar physiological stress response. The noise stimulus used for training, as well as the light, restraint, and tail-pinch stimuli, were presented to groups consistently in type, intensity, and frequency, though were found to produce different responses in the CE and CNE groups. Since both the CE and CNE groups were initially subjected to opposite schedules of operant reinforcement, we can conclude that the groups adapted their eating behaviour during exposure to these stressors according to how feeding had been associated with the noise stressor in the past. Pleasant past associations between eating and stress (i.e. the removal of a stressor as in

negative reinforcement training), resulted in increased eating during future stressful events, whereas unpleasant past associations between eating and stress (i.e., the presentation of a stressor as in punishment training) resulted in decreased eating. With the discussed results, the present study therefore contributes to literature examining the effects of various types of acute stressors on untrained rats through examination of Control group behaviour and CE and CNE behaviour further propose that learning history, specifically an operant learning model, can affect how rats will direct their eating behaviour during future stressful events.

In light of recent research, the implications of food palatability are especially important to consider when interpreting results of this study due to the use of sugar pellets in training and testing. The aversive state reduction hypothesis and reward-based model of stress eating, each propose that access to sweet foods will more likely cause increased eating in response to stress and that consumption of these foods consequently reduces activation of the stress response (Adam & Epel, 2007; Pool et al., 2015). Therefore, rats who ate more while exposed to stressors in testing (expected by the CE group), may have consequently experienced a reduction of stress compared to rats who consumed less sugar pellets, meaning that groups may have experienced different levels of stress-severity during testing. Although speculative, this could offer an explanation for why the CNE group demonstrated significantly reduced feeding behaviour by the fourth training trial, while divergent feeding behaviour between the CE and Control groups until later trials; if stress experienced by the CE group was reduced consequently following the consumption of sugar pellets during trials, the noise stress would be a less effective punisher in the context of negative reinforcement during punishment training received by the CE group. Results of the restraint test could also possibly be explained by aversive state reduction theories, as similar feeding between CE and Control groups during this emotional stressor could reflect

that increased sugar consumption by these groups consequently evoked a pleasurable experience for subjects, inhibiting the effect of increased feeding by the CE group following the restraint stress (Kinzig et al., 2008).

These theories could also explain the apparent greater impact that training had on the CNE group compared to CE group feeding, as they suggest that higher consumption of sugar by the CE group should result in a reduction of the unpleasant stress experienced by this group during training and testing (Pool et al., 2015). However, this was not directly tested and measuring hormones and neurotransmitters involved in both stress and reward responses such as corticosterone and dopamine, could have further informed these theories and offered clarification for whether sugar consumption correlated an with an inhibited stress response, supporting the role of reward the reward system in these responses (Adam & Epel, 2007). The use of sugar pellets in training and testing, may therefore have influenced results due to the implications of food palatability increasing the likelihood of increased feeding during stress.

Limitations and Future Directions

While this study successfully demonstrated that both increased and decreased feeding in response to stress can be learned by rats through operant conditioning, proposing this as a factor contributing to these effects observed within the human population, the present design had various limitations. The analysis of observed behaviours known to correlate with stress provided limited comparisons of stressor severity or intensity, as few significant differences were reported for these measures (Table 2) and physiological correlates of stress such as corticosterone levels were not obtained. The use of an animal model in a laboratory setting further limits what general conclusions can be drawn from results and to what extent they can be applied to understanding the eating behaviours of humans in a naturalistic setting. Finally, as discussed, implications of

food palatability are important to consider when interpreting results of this study due to the use of sugar pellets. It is therefore important to consider results in the context of past research and theories to understand the implications of these limitations and offer direction for future investigations.

Analysis of freezing, urination, defecation, and grooming behaviours during exposure to stressors indicate that freezing frequency was the only measure consistently affected by exposure to all stress stimuli compared to the absence of stress. The main effect of stressor-type identified increased freezing during the noise, light, restraint, and pinch tests, compared to the no-stress test, and freezing was further increased during the light test compared to all other tests. Consistent with these results, the light was the only stressor to affect the feeding behaviour of the Control group and appeared to have the greatest impact on the feeding behaviour of CE and CNE groups aside from the noise stressor used to train them (Figure 4). The large effect of the noise test on CE and CNE group eating can be explained by direct associations made with the noise during training, while the magnitude of effect of the light stressor on feeding could imply that it was more effective than restraint and pinch stressors at inducing a stress response, or that the light stressor was perhaps more effective than restraint and tail-pinch stressors at causing rats to experience a stress response similar to that caused by the noise stress, resulting in more generalization of learned responses between the noise and light stimuli. Limited significant effects observed for other behavioural measures could have been influenced by the small sample size of groups as the repeated-measures ANOVA comparing behavioural measures by groups for all tests, had a low power and effects may have been observed if more subjects had been assigned to each group.

Though behavioural correlates of stress were not consistently observed, this study was further limited as it did not include measures of physiological correlates of stress such as corticosterone and other hormones and neurotransmitters involved in the stress response that may have allowed comparisons of the intensities of the stressors that were tested. As discussed, CRH, ACTH, and corticosterone levels, are all affected during stress (Adam & Epel, 2007; Dickerson & Kemeny, 2004; Maniam & Morris, 2012) but measures for these were not obtained. Due to these limitations, the stress-response of subjects during noise, light, restraint, and pinch tests cannot be quantified or compared. Previous research has however observed increased corticosterone levels and behavioural measures indicative of stress by rats in response to exposure to these types of stressors and have used similar stimuli as stressors in laboratory investigations. Though increased freezing frequency was the only significant observation supporting that testing stimuli were effective at causing stress, previously discussed literature further supports this assumption.

In future research, different stressors could be investigated within the present paradigm with additional physiological measures to gain appropriate and necessary representation of typical response sets resulting from schedules of negative reinforcement and punishment training. This new training paradigm could be further used to explore the strength of training by testing how long differences in feeding behaviour would persist under stress and at what point these differences would extinguish from the subjects' behavioural repertoires, past the 10 day period prefacing the present follow-up tests. Future studies should continue to investigate the influences of stressor type, intensity, and frequency on feeding responses discussed frequently in past literature, as they have been examined inconsistently between studies and it remains unclear what qualities of stressful stimuli will determine how feeding will be affected (Greeno & Wing,

1994). While implications of stressor-type must further examined, implications of food-type should further be examined within the context of the proposed model due to the relevance of food palatability in recent investigations (Maniam & Morris, 2012). It is impossible to know without further investigation, whether operant training would have been effective if highly palatable food had not been selected to be used in training, nor is it clear whether learned feeding responses to stress would persist if a different food were offered than the sugar pellets used in training. Future research to investigate the effects of food palatability within the present design would contribute to understanding the implications of food palatability and learning history on feeding responses to stress.

Finally, the use of a controlled animal model limits the extent to which conclusions drawn from this experiment can be applied to understanding human behaviour. The use of rats also prevents subjective reporting of stress experienced that could be obtained in a human study through self-report measures. Since physiological correlates of stress were not measured, behavioural observations were the only data collected to support whether stimuli were effective in causing stress to be experienced by subjects (with only freezing frequency demonstrating consistent significant effects). While this study demonstrates that operant learning can produce increased or decreased eating responses by rats exposed to stress, it is unclear exactly how this learning may manifest in naturalistic settings. Attempts to replicate this experiment in human subjects would be difficult because it would be impossible to maintain the same level of control over extraneous variables outside of testing and because human subjects may have already developed a stress eating or non-eating response to stress.

Self-report by individuals who already demonstrate stress eating and non-eating responses may however allow for past experiences of associating stress and changes in feeding

behaviour to be identified retrospectively to support this theory in humans. Learning paradigms have been effective in producing changes in the feeding behaviour of individuals in human studies, notably, operant learning has been effective in treating anorexia nervosa in human subjects, though is most effective in short-term treatment to increase feeding and weight (Bemis, 1987; Bhanji & Thompson, 1974; Touyz, Beumont, Glaun, Phillips, & Cowie, 1984). Recent research has further identified implications of learning theories on obesity, proposing that overeating by obese individuals promoted by behavioural and environmental cues associated with overeating in the past, may be consequently reduced through mechanisms that enhance extinction of these cues (Boutelle et al., 2015; Boutelle & Bouton, 2015). Considering these examples and the results of the present study, it may be possible to apply learning theory to developing programs aimed at changing individual's existing eating patterns in response to stress, either through the conditioning of new associations between stress and feeding, or by encouraging the extinction of existing associations that have be reinforced in the past. These types of interventions could be beneficial in cases where eating behaviour changes during stress may contribute to health conditions such as obesity and eating disorders.

Conclusion

An issue in research in this domain will undoubtedly be associated with the need for the execution of strictly controlled human studies of the nature of the present study for the various findings related to stress eating and non-eating in rats to be applied to the human population. Still, the results of the present study along with the suggested improvements and described recommendations for future research, contribute to the domain of feeding research and contribute to the search for an accurate scientific explanation for changes in eating behaviour during exposure to stress, demonstrating for the first time that stress eating and stress non-eating may be

conditioned responses as these responses were found to be influenced by past operant learning. It is important to note that past research has investigated the effects of multiple variables on the stress-eating relationship, such as individual differences in weight and dieting history, as well as differences in food composition and stressor-type (Adam & Epel, 2007; Greeno & Wing, 1994). While research has not described consistent effects of these variables, there are clearly multiple factors influencing how stress will affect an individual's eating during stress, and it is unlikely that a single theory will account for a full explanation of these effects. While many variables are likely involved in determining how an individual will direct their eating response during stress, operant learning produced divergent feeding responses to stress in a controlled study, suggesting that in a naturalistic setting, inadvertent associations made between increased stress and changes in feeding behaviour that occur repeatedly, may influence future responses of stress eating and non-eating. Considering that the high prevalence of reported stress by individuals that continue to escalate in this society (2007, American Psychological Association) along with the comorbidity of health problems associated with stress such as obesity, eating disorders, and risk of addictive behaviours (American Psychological Association, 2010), research in the domain of stress and eating behaviour may be important to our understanding of and in developing interventions to manage the implications of these behaviours on the health of individuals.

APPENDIX A

Time Line of Methods:

(Arrival to colony) (Group assignment) (3 day break)

I	I	I	I	I
Orientation	**Habituation**	**Conditioning**	**Noise test**	**No-Stress test**
- 10 days in home cages	- 7 trials; 10 subject trials/day (21days)	- 9 trials/group (42days)	- 1 day/group (3days)	- 1 day/group (3days)

I	I	I	I
Reconditioning/ Light-Shock association trials	**Light Test**	**Reconditioning**	**Restraint Test**
- 3 reconditioning trials/group (9days) - Separated by 2 days of light-shock association for all groups (2days)	- 1 day/group (3days)	- 3 trials/ group (9days)	- 1 day/group (3days)

(10 day break) (End)

I	I	I	I	I
Reconditioning	**Pinch Test**	**Reconditioning**	**Follow up No-Stress test**	**Follow up Noise test**
- 3 trials/ group (9days)	- 1 day/group (3days)	- 3 trials/ group (9days)	- 1 day/group (3days)	- 1 day/group (3days)

APPENDIX B:

ACC Ethics Approval:

TO: Dr. Michael Emond – Department of Psychology

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

DATE: April 10, 2015

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

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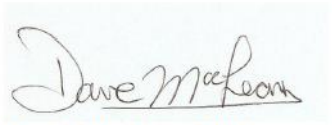
Dr. Emond,

On April 8, 2015, 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

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