AN INVESTIGATION OF THE EFFECT OF VICARIOUS SOCIAL DEFEAT STRESS ON STRESS HORMONES AND FOOD CONSUMPTION IN MALE MICE.

by

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Abstract

In addition to causing depressive-like behaviours in rodents, stressors can also alter feeding behaviour and body weight. The current study aimed to investigate the vicarious social defeat stress model as a means of studying the effects of stressors on feeding, in a manner that allows for comparison between directly affected victim animals and witnesses to social defeat. Increased corticosterone secretion occurred in both acutely treated victim and witness animals 10 minutes following stressor exposure, an effect that persisted in the victim animals for 1.5 hours. No significant effects were found in chronically treated groups, which may be the result of a habituation-like effect to repeated stressor exposures. No significant changes were found in food consumption, weight gain, or plasma ghrelin. Overall, these findings shed light on the impact of social stressors and the consequences of being involved in, compared to witnessing, social defeat.

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Introduction

Obesity is a condition of excess body fat and is associated with an increased risk of cardiovascular disease, high blood pressure, type 2 diabetes, stroke, and arthritis. It is also associated with a higher incidence of depressive illness. Obesity was estimated to affect 2.1 billion people worldwide, with obese individuals having a two-fold increase in likelihood of developing depressive illness (Roberts et al., 2003).

The link between obesity and depressive illness suggests that environmental factors that are stressful, and therefore associated with mood disorders like depression, may also be associated with the development of obesity (Razzoli et al., 2017). In support of this notion, there are data showing that stressors elicit numerous physiological changes that facilitate the mobilization of resources to overcome the stressors' negative actions and maintain homeostasis, a concept referred to as allostasis (Sterling and Eyer, 1988). However, prolonged exposure to stressors, and continuous allostatic responses to deal with the stressors, can have maladaptive consequences including alterations in brain functioning, as well as numerous behavioural and psychological consequences (Lupien et al., 1998). Likewise, prolonged stressor exposure may promote neurometabolic alterations that can lead to weight changes (Nephew and Bridges, 2011).

Stress circuitry: the hypothalamic-pituitary-adrenal axis

The circuitry most heavily involved in regulation of stress responses is the hypothalamic-pituitary-adrenal (HPA) axis. Stressful stimuli cause the release of

corticotropin-releasing hormone (CRH) and vasopressin from the paraventricular nucleus of the hypothalamus, which in turn promotes the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, which causes the release of glucocorticoids such as cortisol in humans and corticosterone in rodents. An increase in circulating glucocorticoids causes a rise of blood glucose to prepare for adaptive defensive responses. The activation of this axis is adaptive and follows a circadian rhythm- highest levels of circulating glucocorticoids are found at the time that an animal typically wakes up, which may help the animal mobilize glucose in preparation for its active period (Chung et al., 2017). However, disruption of this rhythm can also be observed as a result of chronic stressor exposure, leading to maladaptive responses on cognition and behaviour. In depressed patients, elevated HPA axis activity is associated with an increase in CRH neurons in the paraventricular nucleus of the hypothalamus (PVN), as well as increased circulating corticosteroids (Gold and Chrousos, 2002).

Effects of HPA axis dysregulation

The hippocampus and HPA axis have been implicated in the development of depressive illness, particularly after exposure to stressors. Glucocorticoid receptors are found in high concentrations in the hippocampus, and excessive or prolonged glucocorticoid receptor activation can inhibit plasticity and hippocampal neurogenesis (Masi and Brovedani, 2011, Dellarole et al., 2014). In healthy animals, glucocorticoid receptor activation leads to inhibition of the hypothalamus to reduce the release of glucocorticoids, but with chronic stressor exposure, this pathway can become damaged, leading to more maladaptive stress responses, as well as

proposed damage to cognition related to the processing of stress and emotion (Paizanis et al., 2007). With such deleterious effects on cognition, it is important to understand glucocorticoid release and regulation, as it may impact stress coping mechanisms in humans.

Glucocorticoids and eating behaviours

Glucocorticoids initially suppress appetite, but are released for an extended period following a stressor, which can lead to an increase in feeding behaviours (Heinricks et al., 1993, Dallman et al., 2003). Three non mutually-exclusive mechanisms have been proposed to mediate this relationship: i) stimulation of neuropeptide Y (NPY) and agouti-related peptide (AgRP) in the arcuate nucleus, which promotes feeding behaviours, ii) glucocorticoid-induced insulin release from the pancreas, which increases preferences for highly palatable foods, and is eventually linked with insulin resistance with long-term exposure (Shimizu et al., 2008, Kamegai et al., 2001), and iii) some studies have proposed that glucocorticoids increase salience of pleasurable stimuli, such as food (Dallman et al., 2003). Each of these mechanisms of action link glucocorticoids, and therefore stress, to energy balance and obesity. Indeed, differential activation of these pathways might explain some inter-individual differences in how an animal responds to stress.

Ghrelin and eating behaviours

Ghrelin, a 28-amino acid peptide secreted primarily by the fundus of the gut, is one of the key hormones involved in food intake and energy balance, as it serves

to promote food intake (Kojima et al., 1999). Plasma ghrelin is typically increased before meals, and decreased afterwards, with particular importance placed on the enzyme ghrelin o-acyltransferase (GOAT), which activates ghrelin by modifying its third amino acid, serine (Kojima et al., 2016). Ghrelin can produce its effects through its receptors- growth hormone secretagogue receptors (GHSR) found in the arcuate nucleus and ventromedial hypothalamus as well as in dopaminergic neurons in the ventral tegmental area. Ghrelin acts on energy balance by increasing caloric intake and reducing fat utilization (Tschop et al., 2000), and it suppresses insulin secretion from the pancreas and promotes glucagon release, promoting the release of glucose into the blood by the liver (Reimer et al., 2003, Chuang et al., 2011).

In addition to its effects on homeostatic energy balance, ghrelin is also associated with reward circuitry that responds to highly palatable food. GHSRs are found on the dopaminergic cells in the ventral tegmental area that project to the nucleus accumbens and the prefrontal cortex, areas associated with reward and goal-directed behaviours (Muller et al., 2015). It is thought that through this neural circuitry, ghrelin can promote food-seeking behaviours.

Ghrelin and stress

Ghrelin's actions are both vulnerable to, and protective against the effects of stressor exposure, as demonstrated by several rodent studies. Plasma ghrelin has been positively correlated with stressor exposure, and has also been linked to HPA axis activity variations. Ghrelin is expressed in hypothalamic CRF afferents, and ghrelin administration has been linked to increased ACTH release from the pituitary (Arvat et al., 2001). In addition, plasma ghrelin levels have been shown to increase

in response to social stressors in both humans and rodents (Raspopow et al., 2010; Rostamkhani et al., 2016). This elevation is sustained in both emotional and non-emotional eaters, which may serve to promote energy intake in order to cope with stressful stimuli.

In rodents, ghrelin contributes to increased food intake in response to social stressors (Sanghez et al., 2013; Razzoli et al., 2015). It is also increased following chronic social stressor exposure, and may prevent the onset of anxiety-like and depressive-like behaviours following a stressor (Lutter et al., 2008). These increased food consumption behaviours have been found to occur through the ventral tegmental area, and may serve to protect against chronic stressors by compensating for energy demand (Skibicka et al., 2011). In other stress models, such as restraint stress, these effects were found to occur through stimulation of the HPA axis, which may utilize the previously mentioned glucocorticoid mechanisms (Spencer et al., 2012). However, prolonged stress and ghrelin activation can also lead to maladaptive food intake and obesity in rodents (Patterson et al., 2013; Razzoli and Bartolomucci, 2016). This observed ghrelin increase is also linked to alterations in brain-derived neurotrophic factor, suggesting that it may play a role in depressive illness and responsiveness to treatment (Razzoli et al., 2011). Thus, ghrelin is a key player in adaptive responses to stressors, but may also mediate some negative consequences of prolonged stress and treatment efficacy.

Chronic stress in rodents

In rodents, chronic social defeat stress leads to numerous behavioural changes, including an increase in social avoidance behaviours (Henriques-Alves and

Queiroz, 2016), anhedonia, weight loss, and an increase in anxiety-like behaviours (Krishnan et al., 2007). Neurometabolic changes, such as increased corticosterone secretion, are also observed in mice exposed to this paradigm (Gong et al., 2015). However, some of these effects abate with repeated stressor exposure. This type of abated response is seen in many contexts and is known as habituation- a reduction in response to a signal after repeated exposures (Bouton, 2007). While still elevated compared to non-stressed controls, chronically stressed mice show blunted corticosterone secretion compared to their acutely stressed counterparts in chronic social defeat stress paradigms (Keeney et al, 2006; Herman et al., 2016, Iredale et al., 1996). The habituation-like effect observed in chronically stressed animals is thought to occur as an adaptive response to minimize damaging effects of chronically elevated glucocorticoids, such as adrenal hypertrophy, hippocampal cell loss, and weight changes (Bowens et al., 2012). Therefore, animals undergoing chronic stress are expected to show reduced stress responses compared to acutely stressed animals.

The vicarious social defeat stress model

The chronic social defeat stress paradigm is used to model human depressive illness in rodents. Most current stress-depression models, such as the forced swim test, restraint stress, and even interactions during chronic social defeat stress involve a physical component, but humans often experience emotional stressors, which can instigate the development or exacerbation of psychiatric illness. However, this may not accurately model all causes of mood disorders in humans. The idea behind the vicarious social defeat stress model was to more closely capture

the types of stressor that humans face in everyday life: we are not typically shocked or restrained in our daily lives, but instead a lot of our stress comes from our negative interactions with other people. The vicarious social defeat stress model in mice promotes depressive- and anxiety-like effects in mice that are reversible with antidepressant administration (Nestler & Hyman, 2010).

An additional advantage of the vicarious social defeat stress paradigm is that it allows us to implement manipulations that remove some of the physical components of a stressor, allowing researchers to better understand the social and emotional components of stressor exposure. In classic stressor paradigms, inflammatory responses may be elicited due to physical injury, confounding the observation of neuroendocrine stress responses (Ramirez and Sheridan, 2016). The vicarious social defeat stress paradigm includes exposures to stressors as in the chronic social defeat stress model, but introduces a group of witness animals that simply observe the bully-victim interactions without ever coming into physical contact with them (Sial et al., 2016). This has shown to produces a reliable stress response in the victim animals comparable to that seen in the victims of the chronic social defeat stress paradigm in terms of depressive- and anxiety-like behaviour, cardiovascular changes, and inflammatory markers, as well as some overlapping responses in the witness animals (Sial et al., 2016, Finnell et al., 2017). This may also capture a wider breadth of human social interactions that cause stress; even if we are not the victims of bullying or overbearing social situations, we are likely to observe them in the world around us. Many studies of human social psychology,

including the discovery of the mirror neuron system, show us that this can cause social stress in humans (Rizzolatti and Craighero, 2014).

Hypotheses

In light of the findings concerning social defeat and witnessing such encounters, the present study will evaluate the effects of social stressor exposure with regards to food intake and body weight, as well as ghrelin and corticosterone secretion. It was hypothesized that victims of social defeat and witnesses of such events will show greater hormonal stress responses than controls, as well as responses related to food consumption. Further, because an adaptation may occur in response to stressors, acutely stressed animals should show a greater stress response than chronically stressed animals.

Methods

Animals

All experimental procedures were approved by Carleton University's Animal Care Committee (ACC) in compliance with the Canadian Council on Animal Care (CCAC) guidelines. 40 CD-1 male retired breeders and 130 eight-week old male C57BL/6 were obtained from Charles River Laboratories (Sherbrooke, Quebec). Animals were housed in a vivarium at 21-25°C on a 12hr light/dark cycle (lights on between 8:00 and 20:00). C57BL/6 mice were singly housed in ventilated cages containing corncob bedding and a nestlet in one room, while CD-1 mice were singly housed in clear polypropylene cages containing corncob bedding and a nestlet in a separate room. All mice were given ad libitum access to food (standard lab chow) and water throughout the study.

Procedure

Animals were given 3 days to become acclimated to the lab following arrival from the breeders, and then baseline body weights and food intake were recorded over seven days. Food intake was calculated by weighing the food each day, and then subtracting the weight of the food from the previous day's weight. After the baseline period, animals were randomly assigned to experimental groups. The weight and food intake of each animal were recorded daily throughout the rest of the experiment.

The experimental paradigm comprised a 2 (chronic versus acute treatment) x 3 (victim, witness, no treatment), x 2 (time of sacrifice; 10 minutes versus 1.5 hour

following stressor exposure). One additional no treatment group was also included, making 13 groups. Each group was composed of 10 male mice.

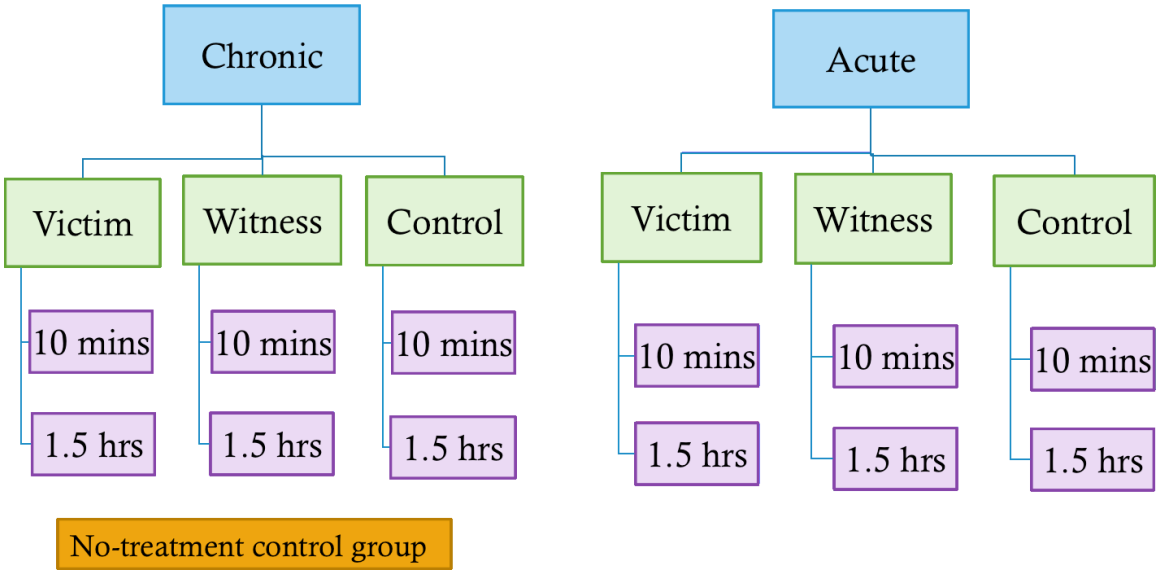

Figure 1: Study design. Each group was comprised of 10 male C57BL/6 mice.

Each victim mouse (C57BL/6) was paired with a bully mouse (CD-1), with which it would interact for the duration of the study. In addition, these pairs were assigned a witness animal (C57BL/6), which would observe all of the victim-bully interactions. The same sets of animals interacted for the entire duration of the study, rather than a novel bully for interaction, based on previous studies wherein the use of the same bully each day successfully promoted a stress response in the subordinate victims (Patterson et al., 2011). Control animals that were not paired with a dominant mouse (i.e., were not bullied) were moved into a novel cage containing corncob bedding but no bully animal, at the same time of day as the victim/witness pairs. The victim/witness pairs, as well as the control groups, were balanced for time of day in order to minimize the confounding effects of time of day

on corticosterone secretion. In the morning, the victim-witness pairs were moved to the room in which the bully animals were housed immediately before the procedure. The bully animal's home cage was divided using a wire mesh screen such that two-thirds of the space was assigned to the bully and victim, and one-third was designated for the witness, so that the witness could observe the bully-victim interaction (see Appendix).

The witness animal was placed in the side of the cage not containing the bully, and the victim was placed in the side containing the bully. The bully and victim mice were allowed to interact until signs of submission (turning over, attempts to escape) were observed. At this point, victim and witness animals were removed and returned to their own cages. In the case that no aggressive or submissive behaviours were observed, animals were returned to their home cages after 5 minutes of interaction. Each interaction was recorded using a video camera. This was repeated for ten days for the chronic groups, and on the last day for the acute treatment groups. The no-bully control groups were moved into the full space of a new cage not containing a bully for five minutes each day in a separate room not containing the bully animals.

Tissue Extraction and Blood Collection

On the last day of testing, animals were sacrificed either ten minutes or 1.5 hours following the procedure. Animals were decapitated and trunk blood was collected and held on ice with ethylenediaminetetraacetic acid (EDTA), an anticoagulant, and methoxyarachidonyl fluorophosphate (MAFP), which inhibits serine hydrolases and proteases in order to preserve serum ghrelin, until plasma

extraction. Plasma was separated by centrifuging blood at 3000 rpm for 15 minutes. Plasma was then removed and stored at -80°C awaiting further analysis. Brains were extracted immediately following decapitation and frozen in 100% ethanol and stored at -80°C. Plasma ghrelin concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits (Millipore) (intra-assay reliability: 1.1-1.7%, inter-assay reliability: 2.0-3.2%). Circulating CORT concentrations were measured using ELISA kits (Enzo Life Sciences) (intra-assay reliability: 6.6-8.0%, inter-assay reliability: 7.8-13.1%).

Statistical Analyses

All data were analyzed using MATLAB R2016b software (Mathworks Inc.). To analyze differences in body weight and food intake, repeated measures ANOVAs were performed with groups based on victim/witness status and stress chronicity compared to controls. To compare differences in both plasma ghrelin and corticosterone concentrations, between-subjects ANOVAs were performed in each measure including all groups. Factors were victim/witness/control status, stress chronicity, and amount of time between the last stressor and sacrificing (3x2x2). All groups were subsequently compared to the no treatment group using Dunnett's test.

Results

Food consumption and body weight

Body weight and 24-hour food intake measurements were taken daily throughout the experiment. After the experiment was concluded, all of the measurements were collected and divided into baseline period and study period weights. Since the acute treatment occurred immediately before the animals were killed, they did not affect the food intake nor body weight measurements, and all of the acute groups were treated as no-treatment controls. In addition, since the sacrifice time (10 minutes or 1.5 hours post treatment) did not affect food intake or body weight, groups were separated only according to chronicity (only chronic groups were considered treatment groups), and social condition (victim, witness, control, and no-treatment control). The treatment groups were therefore composed of 20 animals, and the no-treatment control group consisted of 70 animals. No significant differences between the groups were observed for either body weight or food intake.

Body weight

A one-way repeated measures ANOVA was conducted on body weights to determine if any of the groups differed during the baseline period ($F_{4,125} = 0.99$, p=0.4171, n=10 animals/group, no significant differences found). Since none of the groups differed, the study period weights were compared using a one-way ANOVA ($F_{4,125} = 0.98$, p=0.4185, n=10 animals/group). No differences were observed in the body weight as a result of the treatment (see Figure 1). Overall, as expected, there was an effect of time on body weight ($F_{15,116} = 116.38$, $p<0.0001$, n=10

animals/group), but there were no significant differences between the groups ($F_{3,116}$ =0.21, p=0.8901, n=10 animals/group) or interaction effects ($F_{45,116}$ =0.86, p=0.7287, n=10 animals/group).

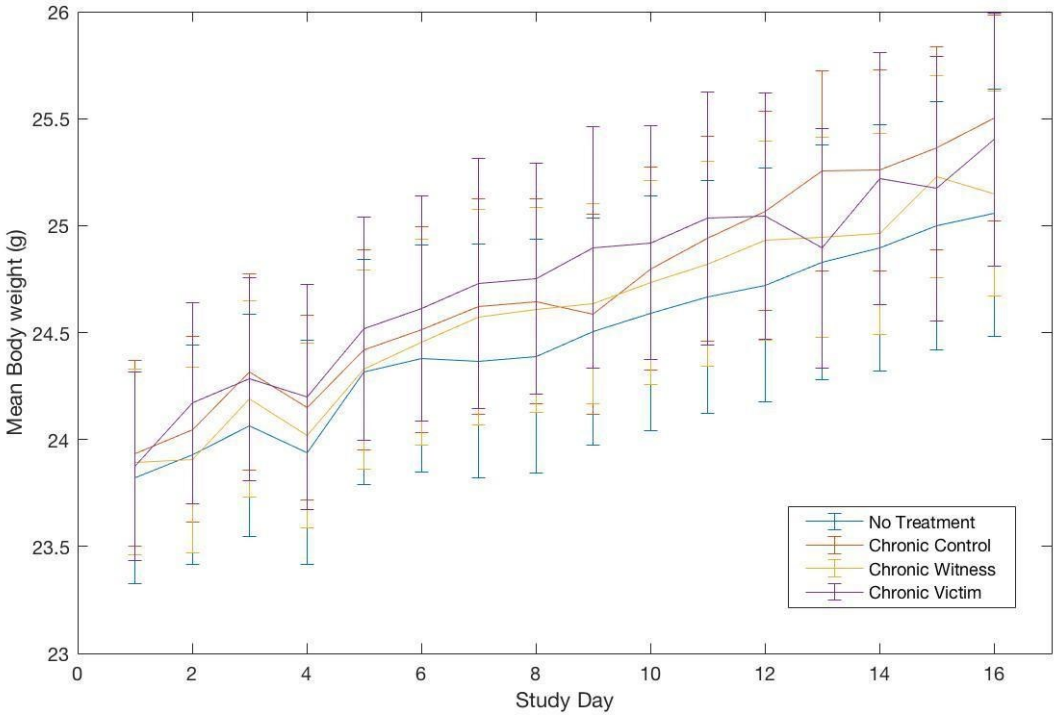

Figure 2. Mean body weight per group at each day during the study (mean+/- SEM). Days 1-7 represent the baseline measurement period, while days 8-16 represent study treatment day. No significant effects of group for either the baseline (p=0.4171) or study period (p=0.4185) were found.

Food Intake

A one-way repeated measures ANOVA was conducted on food intake values to determine if there were any differences between the groups during the baseline period ($F_{4,125}$ = 1.6, p=0.1794, n=10 animals/group, no significant differences). Since the groups did not differ, another one-way ANOVA was run to compare the groups during the study treatment period ($F_{4,125}$=1.36, p=0.2520, n=10 animals/group, no

significant differences). The treatment did not appear to elicit any differences in food intake for any of the groups. There was no difference between groups (see Figure 2). Overall, there was an effect of time on food intake ($F_{15,116}$ = 4.79, p<0.0001, n=10 animals/group), but there were no significant differences between the groups ($F_{15,116}$ =1.9, p=0.1333, n=10 animals/group) or interaction effects ($F_{45,116}$ =0.7, p=0.9333, n=10 animals/group).

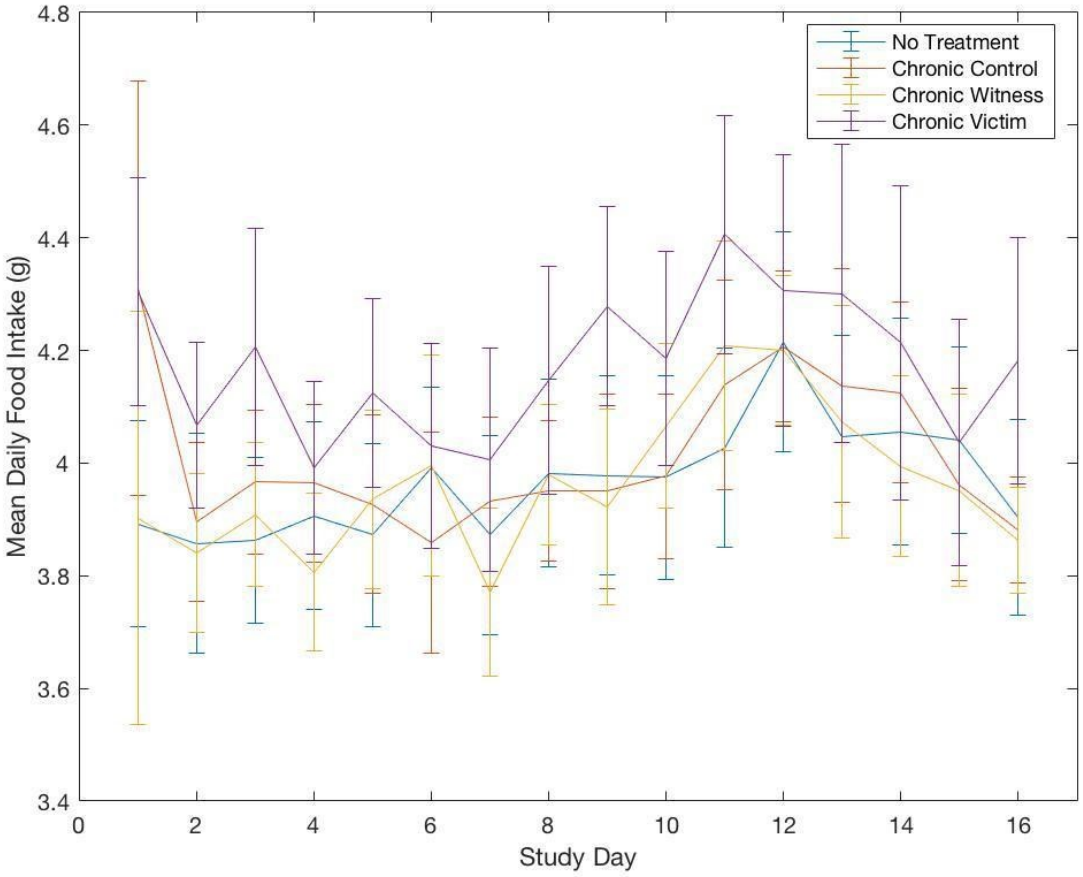

Figure 3. Mean daily food intake per group over the duration of the study (mean+/-SEM). Days 1-7 represent the baseline period, while days 8-16 represent the study treatment period. No significant differences were found during the baseline (p=0.1794) nor the study treatment period (p=0.252).

Hormonal Analyses

At the end of the study, animals were killed by decapitation and trunk blood was collected and stored for analysis of plasma ghrelin and corticosterone. These hormones were analyzed using ELISA kits and 3x2x2 ANOVAs were conducted to analyze the data. Since the treatment of these groups did differ at the end of the study, all groups were treated as separate groups with n=10 animals per group. The no-treatment control group was compared to each of the treatment and control groups using Dunnett's test.

Ghrelin

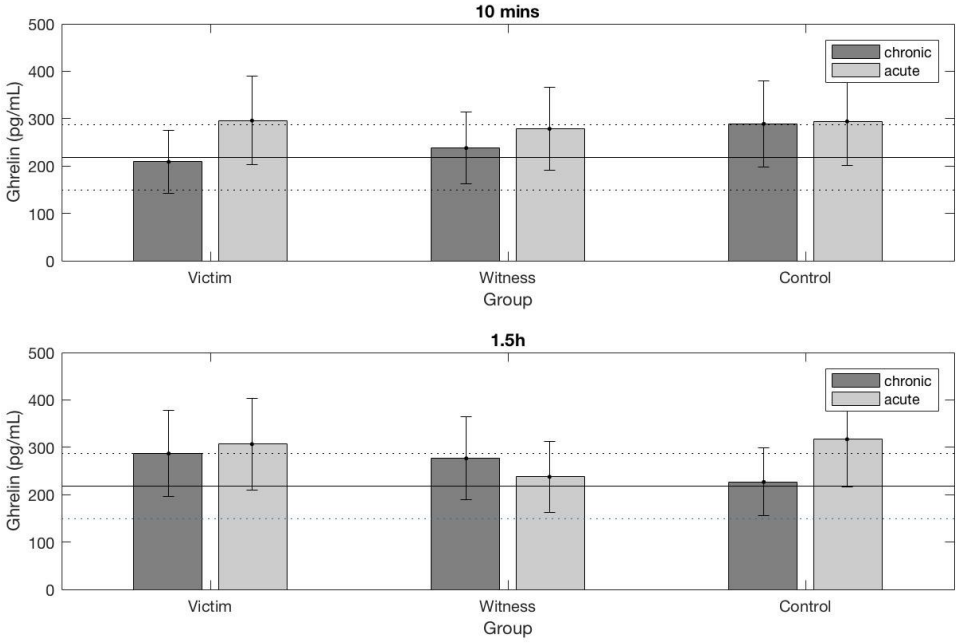

Figure 4: Plasma ghrelin concentration per group at 10 minutes (top) and 1.5 hours (bottom) following treatments, compared to the no treatment control group (black line).

The 3x2x2 (social condition (victim, witness, control) x chronicity (chronic, acute) x sacrifice time (10 minutes, 1.5 hours following treatment)) ANOVA showed

no significant effect of social condition (victim/witness/control) ($F_{2,102}$ = 0.14, p= 0.8716), chronicity (chronic/acute) ($F_{1,102}$ = 0.78, p=0.379), sacrifice time (p=0.8393), social condition and chronicity ($F_{2,102}$ = 0.04, p=0.8314), social condition and sacrifice time ($F_{2,102}$ = 0.25, p=0.7821), chronicity and sacrifice time ($F_{,102}$ = 0.07, p=0.7899), or 3-way interactions ($F_{2,102}$ = 0.49, p=0.6147). Outliers which were more than 2 standard deviations from the mean were removed from these analyses- a total of 5 in this analysis.

Corticosterone

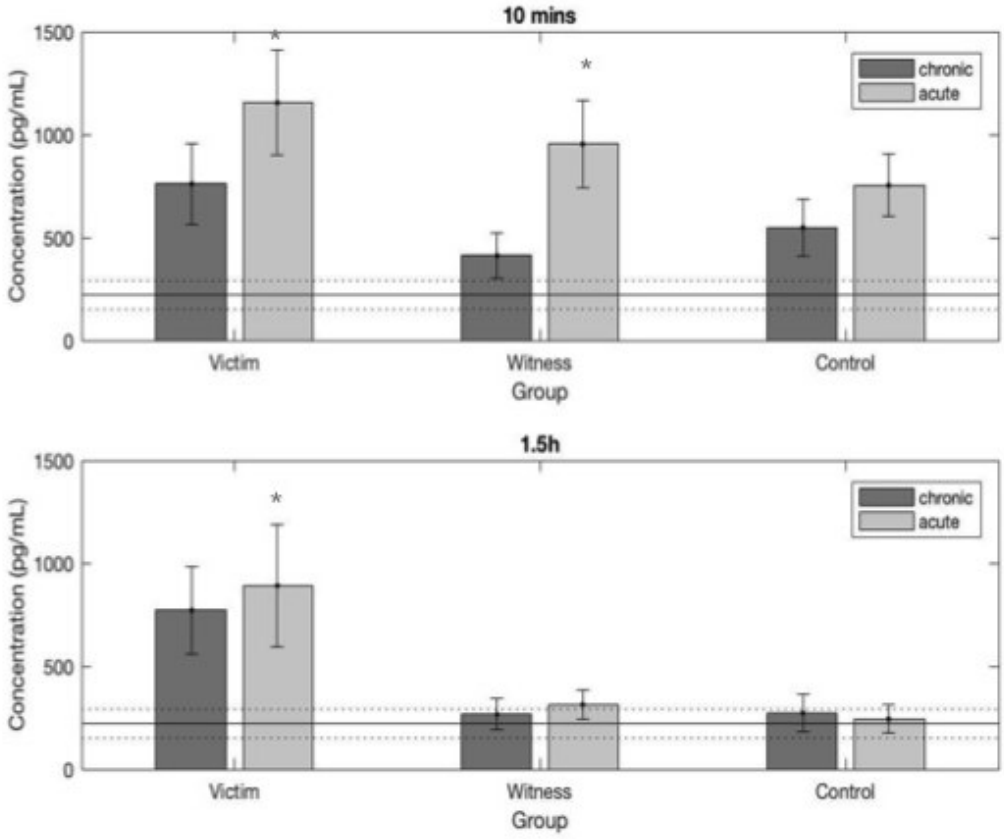

Figure 5: Plasma corticosterone concentration per group at 10 minutes (top) and 1.5 hours (bottom) following treatments, compared to the no treatment control group (black line).

The analysis revealed that corticosterone changes were more pronounced after acute rather than after the chronic stressor ($F_{1,98} = 4.26$, $p=0.0417$) and were also more pronounced 10 minutes and 1.5 hours following stressor treatments ($F_{1,98} = 8.74$, $p=0.0039$). In addition, there was a significant effect of victim stress compared to controls. Follow up tests revealed that victims showed significantly greater corticosterone increases in the witness and control groups, which did not differ from one another ($F_{2,98} = 7.22$, $p=0.0012$). Outliers which were more than 2

standard deviations from the mean were removed from these analyses- a total of 7 in this analysis.

In order to compare all of the groups to the no-treatment controls, Dunnett's tests were performed. Compared to the no-treatment controls, the acute victims and witnesses sacrificed at 10 minutes following treatment, as well as the acute victims sacrificed at 1.5 hours had significantly elevated corticosterone levels ($p=0.0005$, 0.0132, and 0.0288, respectively).

Discussion

Body Weight

Animals were weighed daily to measure changes in body weight. As expected, all of the groups demonstrated an increase in body weight between the baseline and study weights, as animals were expected to gain weight over time, though they were not significantly different from one another. There were no significant differences found in body mass changes from baseline, nor cumulative body weights between groups, and these data do not support the hypothesis that body weight would increase in chronically stressed victims or witnesses, nor the hypothesis that victims would display more profound stress responses. In previous studies, chronic socially stressed animals appeared to undergo a ghrelin-mediated increase in body mass (Patterson et al., 2013), and it was expected that the victims of social defeat in this experiment would demonstrate similar body mass increases. However, not all types of stressors evoke the same response- for example, chronic restraint stress has been shown to lower body mass compared to controls (Jeong et al., 2013, Krahn et al., 1990). Differences in the direction of body mass changes due to the stressor could not explain the current results though, as all animals in a given group received the same stressors but still showed no reliable change in one direction or the other. One more possibility is that inter-individual differences cause some animals to gain weight, while others lose weight or show no changes in response to a stressor. This seems unlikely as all animals were genetically identical, but this is still a possibility that could be formally explored in future work.

The mostly likely explanation for lack of a body mass difference between groups may be related to the potency of the stressor in this model; while these animals were subjected to the same sort of social defeat encounters, some differences exist between the current methods and those used in previous studies. Most notably, the victim animals were not housed with the bully animals, unlike some previous studies, which utilized the chronic social defeat stress paradigm (Patterson et al., 2013, Coccurello et al., 2017). Though this more closely models most human interactions, the reduced exposure used in the current study would very likely decrease the overall potency of the manipulation.

Food Intake

Throughout the baseline and treatment periods, 24-hour food intake was measured for each animal. No significant differences were found between groups or across time for either the average daily or cumulative food intake, which do not support the hypothesis of a change in food intake in response to a chronic social victim stressor or witness stressor. Studies measuring the effect of the vicarious social defeat stress model on body weight and food intake are limited, but other studies using the chronic social defeat stress paradigm show an increase in food intake following chronic social defeat stress treatment (Kumar et al., 2013, Lutter et al., 2008), suggesting that as in the body weight findings, the vicarious social defeat stress paradigm used here may not be sufficient to elicit changes in food intake. However, as with body weight, different types of stressors evoke different changes to food intake- food intake is reduced as a result of restraint stress, as well as foot

shock stress (Jeong et al., 2013, Krahn et al., 1990, Sekino et al., 2004, Kuriyama and Shibasaki, 2004).

Ghrelin

Plasma ghrelin concentrations were compared based on chronicity, social condition, and sacrifice time. No significant effects were found in any factor. However, other studies demonstrate a change in ghrelin secretion as a result of stressor exposure (Chuang et al., 2011, Kumar et al., 2013). The absence of significant effects with respect to ghrelin secretion, as well as food intake and body mass, may once again reflect reduced potency of the stressor. Corticosterone secretion was altered as a result of the experimental manipulations, but this may be a more sensitive measure than those observed in metabolic processes. When compared to results of chronic social defeat stress studies, it appears possible that the vicarious social defeat stressor used in this study may not be potent enough to produce metabolic changes because the animals are not housed together as they were in other social defeat paradigms (Sial et al., 2016, Warren et al., 2013, Tsankova et al., 2006, Berton et al., 2006). Another possibility is that effects on ghrelin, food intake, and body mass might have been seen if the animals were exposed to chronic stressors over a longer period of time, or with a new bully animal for each interaction, as studies with longer stressor schedules and novel bullies showed changes in those measures (Razzoli et al., 2015, Henriques-Alves and Queiroz, 2016).

Corticosterone

Plasma corticosterone was assessed and compared in groups according to chronicity, social condition, and sacrifice time. The acutely stressed victims displayed an elevation in plasma corticosterone at both 10 minutes and 1.5 hours following treatment. This indicates a prolonged increase in corticosterone secretion similar to those previously observed (Sial et al., 2016). Acutely stressed witnesses also demonstrated an elevation in plasma corticosterone, though this elevation was only statistically significant in the groups sacrificed at 10 minutes following stressor exposure. This indicates an increase that lasted less than 1.5 hours following stressor exposure, in contrast to the prolonged elevation observed in the acute victim group. The prolonged elevation of plasma corticosterone in the acute victims, but not the acute witnesses, indicates that the actual social defeat may be more stressful than simply witnessing the encounters. The corticosterone effects observed at 1.5 hours may result from a greater stress experience, requiring a longer time for the animal to return to baseline corticosterone levels, which may be the result of a more stressful experience.

The chronically stressed groups did not display any significant changes in corticosterone secretion. This was expected, given the previously observed habituation-like effects seen in chronically stressed animals (Bowens et al., 2012, Boleij et al., 2014). As mentioned previously, a dampening of the stress response observed during repeated stressor exposures might serve as an adaptive response, enabling the animals to cope with the stressor, but is also associated with depressive-like phenotypes (Thompson and Spencer, 1966, Eisenstein and

Eisenstein, 2006, Bowens et al., 2012). However, in a similar study, chronically stressed animals in all groups appeared to have a greater corticosterone secretion than those that were acutely stressed (Sial et al., 2016). Likewise, Warren et al. (2013) demonstrated an increase in corticosterone secretion in chronically stressed victims and witnesses as well (Warren et al., 2013). These studies involved housing the victims with the bully animals, while ours did not. It is possible that the longer-term exposure to the bully animals may account for the larger increase in corticosterone secretion seen in chronically treated groups. Beyond that, it may simply be a lack of power that could have to detect elevated cortiosterone in response to the chronic social defeat stressor.

Previous work has suggested that stressors with a physical component, such as those experienced by the victim animals, promote a greater stress response than those without a physical component, though both are indeed stressful for the animals (Warren et al., 2013). This is demonstrated in the current study by the duration of the corticosterone elevation in the acutely treated victim group, compared to the shorter duration of this elevation in the acutely treated witnesses. Other studies have revealed similar findings with respect to stressor severity. A study on cardiovascular consequences of this stress paradigm revealed differences in both victim and witness groups (Finnell et al., 2017). Exposure to this paradigm resulted in similar increases in heart rate and mean arterial pressure in both the victim and witness groups, while only the victims showed stress-induced arrhythmias. The extent to which these effects are caused by physical contact versus

having a more potent stressor is, however, not clear. Current studies, including the one presented here, cannot disentangle these effects because physical contact itself could lead to greater stress responses even in the absence of an inflammatory response. However, current and previous work does provide some evidence that there may be differential effects of physical versus psychological stress.

Limitations and Future Directions

This study demonstrated the differential effects of being a victim versus being a witness of a social defeat stress encounter, as well as differences resulting from acute and chronic stressor exposures. However, some limitations should be noted. For one, the magnitude of the stressor could be increased by housing the animals together, as in chronic social defeat stress models, in order to determine if some effects of the stressors were undetectable because of reduced potency. Future studies could incorporate a longer-lasting stressor, or a different housing structure, similar to that outlined by Golden et al., in order to further verify the effects of this type of stressor on metabolic pathways (Golden et al., 2011). As well, given that repeated interactions between the bully and the victim animals reduced latency to submissive behaviours, the interactions were eventually reduced to less than ten seconds each. This may not be long enough to reliably expose witness animal to a stressful stimulus, and future studies should incorporate some means to verify that the stressor is sufficient. One of the aims of the current study was to differentiate between physical and non-physical stress, in an attempt to further understand common human stress-related pathology. However, because the experiment was carried out in rodents, the results of this study and any study carried out in rodents

are limited in their potential to translate to human behaviour, given the complexity of human social interactions and individual variability. The stress response of rodents is thought to be similar to that seen in humans, but it is not clear whether the relative strength of a physical compared to nonphysical stressor is similar across these species.

Conclusion

Taken together, the results from this study suggest that both being a victim of social defeat, as well as witnessing these encounters, is stressful for an animal, though being the victim results in a longer lasting stress response. This is in line with our hypotheses, as the victims are not only directly involved, but are also in physical contact with the bully animals. In addition, an acute exposure to these social stressors produces a greater stress response than a chronic stressor, as seen in the plasma corticosterone levels, which supports the notion of a habituation-like effect of repeated stressor exposure. This vicarious social defeat stress paradigm does not provide a conclusive link between stressor exposure and increased food consumption behaviour, but in light of current literature, a modification of this paradigm may prove useful in investigating this link in future work.

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Appendices

Appendix A: Bully-Victim Interaction Setup