Long-term behavioral and neuroendocrine consequences of early adversity (juvenile stressor exposure), and the buffering effects of 'comfort' food.

by

Jennifer Christine MacKay, BSc

Thesis submitted to the Faculty of Graduate and Postdoctoral Studies

in partial fulfillment of the requirements

for the degree of Doctorate in Philosophy in Clinical Psychology

School of Psychology

Social Sciences

University of Ottawa

Abstract

The adolescent period has been proposed to be exquisitely sensitive to the impacts of stress and juvenile stressor exposure is associated with anxiety- and depressive- like characteristics in adulthood. Among adult rats, access to a palatable diet has shown to mitigate the effects of stressors, a form of 'self-medication.' The present collection of studies sought to further characterize the long-term consequences of stressor exposure early in the juvenile period, as well as the use of palatable food as a coping strategy. The first study (Chapter 2) highlighted the importance of methodological rigor in the design of experiments employing social stressors. The second study (Chapter 3) provided further evidence that exposure to juvenile social defeat can have long-lasting consequences into adulthood, and access to a palatable diet may impart some resilience to initial stressor exposure. The third study (Chapter 4) demonstrated that access to a palatable diet can mitigate the long-term behavioral consequences of a 3-day sub chronic non-social stressor applied during juvenility in pair housed rats. The fourth study (Chapter 5) sought to replicate these findings in individually housed (purportedly more stressed) animals. Interestingly, access to a palatable diet was sufficient to protect against the neuroendocrine consequences of juvenile stress but did not mitigate the behavioral consequences, raising the question of an effectiveness "threshold" of self-medication via a palatable diet. The final study (Chapter 6) provided some preliminary evidence that exposure to juvenile stress amid access to a palatable diet has long-lasting changes on dopamine receptor expression in the nucleus accumbens, although the functional significance needs further characterization. Collectively, all studies provided further evidence that self-medication with a palatable diet comes at the price of

poor metabolic outcomes. The results of this body of work provide further evidence that exposure to stress during juvenility can have protracted effects into adulthood, at the cost of poor metabolic outcomes. It also raises the suggestion of an effectiveness threshold of palatable food to cope with stress. Further understanding of the interplay between stress and diet may serve to inform the development of prevention based programs to mitigate the rising tide of concurrent childhood obesity and levels of perceived stress.

Dedication

This thesis is dedicated to:

The loving memory of my mother, Lucie Pilon-Mackay, whose courageous battle with cancer inspired me to pursue a career in healthcare.

To my wonderfully supportive father Brain MacKay and step-mother Lyette Babin-MacKay whose love and generosity helped me navigate the difficult times and encouraged me to put forth my best

Acknowledgments

The journey through a Doctoral Program in Clinical Psychology is a long road full of both wonderful and happy moments, as well as those stressful times where you want to pull your hair out. This is not a road to be travelled alone and I have many wonderful people who have helped me along the way.

First, I would like to thank my thesis supervisor, Dr. Zul Merali, who welcomed me into his laboratory while I was completing my undergraduate degree. This brought me into the world of research and introduced me to some amazing mentors. Zul, you provided me with the fundamental supplies needed to travel this road, including the means to carry out my research, the structure to keep me on track and the ideas and inspiration to help shape the path that my research took. It has been pleasure working with you over the past nine years.

I am forever indebted to Dr. Pamela Kent, whose dedication and commitment to training young researchers guided me throughout this journey. Pam, you are an essential ingredient in the laboratory. You have tirelessly supported me throughout this dissertation on both a professional and personal level through talking out ideas, problem solving roadblocks, reviewing and editing countless manuscripts and offering me an empathetic ear to share my frustrations. I will never be able to express enough gratitude for all that you have done. Thank you for everything.

I am deeply grateful for the support of Dr. Catherine Kyeremanteng, who inspired me to pursue my PhD in clinical psychology. Cathy, you are a mentor and a dear friend. There are not enough words to thank you for all the advice, time and support you provided me with over the past nine years. Having someone to turn to during the bumps on this journey has

meant the world to me. I feel so grateful and privileged to have worked alongside such an amazing woman and clinician.

There were numerous students, employees, and volunteers who contributed to the work that made up this dissertation. However, there are a few individuals whom were crucial to the success of the studies presented in this dissertation. Jon James, you were indispensable cog in this process and went above and beyond the call on countless occasions. Thank you for your attention to detail, expertise, and curious mind which helped investigate and troubleshoot the various hurdles along this journey. I have thoroughly enjoyed the countless hours we have spent working together and the friendship we have shared. Your support and humour have contributed to my journey through this program more than you know.

Samantha Graitson and Eliza Ali contributed substantially to this work: running experiments alongside me (and even sometimes without me), entering data, reviewing videos, processing samples, and offering unique insights and expertise. Ladies, it was a pleasure to work alongside you, pass on my knowledge and watch you achieve the goals you have set for yourselves. Christian Cayer, there would be no social interaction data in this dissertation without you. Thank you for your contributions, ideas and support over the years. I am also thankful to Dr. Marie-Claude Audet, who taught me the ins-and-outs of qPCR and shared her experience of graduate school with me. Thank you for reminding me that there is a light at the end of the tunnel.

Lastly, my journey through this program and dissertation was greatly supported by my wonderful family, partner and friends. A special thank you to Brian MacKay, Patrick Gaffney and Christina Watts for the many hours they spent listening to me vent my frustrations, and helping me take breaks from work when needed.

Table of Contents

List of Figures

Chapter 2.

Chapter 3.

Chapter 4.

Chapter 5.

List of Tables

Chapter 2.

Chapter 3.

Chapter 6.

List of Abbreviations

ACTH	Adrenocorticotropic releasing hormone
ANOVA	Analysis of variance
ACC	Anterior cingulated cortex
ANS	Autonomic nervous system
AVP	Arginine vasopressin
Actβ	β-actin
BMI	Body mass index
CIHR	Canadian Institute for Health Research
CMS	Chronic mild stress
CVS	Chronic variable stress
cDNA	Complementary deoxyribonucleic acid
CRF	Corticotropin releasing factor
C_Q	Cycle quantification value
D_1	Dopamine receptor 1
D_2	Dopamine receptor 2
D_3	Dopamine receptor 3
DA	Dopamine
EPM	Elevated plus maze
FST	Forced swim test
GR	Glucocorticoid receptor
HPA	Hypothalamic-pituitary-adrenal
IP	Intraperitoneal injection

MR	Mineralocorticoid receptor
mRNA	Messenger ribonucleic acid
NAc	Nucleus accumbens
OF	Open field test
PVN	Paraventricular nucleus of the hypothalamus
PD	Post-natal day
PFC	Prefrontal cortex
qPCR	Quantitative polymerase chain reaction
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RPL19	Ribosomal protein L-19
SEM	Stand error of the mean
SI	Social interaction test
VBS	Visible burrow system

Preface

All of the experiments presented henceforth were conducted in the Psychopharmacology Laboratory at the University of Ottawa's Institute of Mental Health Research in Ottawa, Ontario Canada. All procedures employed in the experiments met the guidelines established by the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Ottawa Institute of Mental Health Research.

I was the lead investigator on all experiments outlined in the subsequent Chapters. I was responsible for all major aspects of concept formation, design and implementation of the experiments, data collection and analysis, as well as in the preparation of the majority of manuscript. Dr. Zul Merali was the supervisory author on all experiments outlined in this dissertation and provided conceptual input and manuscript edits. Dr. Pamela Kent provided conceptual input and contributed to data analysis and manuscript edits for all Chapters. Mr. Jonathan James and Mr. Christian Cayer provided support with data collection and neurochemical analysis for all Chapters. In Chapter 2, Drs. Zach Patterson and Alfonso Abizaid provided conceptual input regarding the design of the stressor paradigm and choice of diet, as well as provided training in the hand dissection of fat pads. In Chapter 4, Dr. Hymie Anisman provided conceptual input and assisted with manuscript edits. In Chapter 5, Ms. Samantha Graitson assisted data collection and manuscript preparation. In Chapter 6, Ms. Eliza Ali assisted with data collection and analysis, as well as some aspects of manuscript preparation. Dr. Marie-Claude Audet made substantial contributions to quantitative polymerase chain reaction analysis used in Chapter 6 and also assisted with manuscript edits.

A version of Chapter 4 has been published [MacKay JC, James JS, Cayer C, Kent P, Anisman H, et al. (2014) Protracted Effects of Juvenile Stressor Exposure Are Mitigated by Access to Palatable Food. PLoS ONE 9(5): e96573. doi:10.1371/journal.pone.0096573] and is licensed under the terms of the Creative Commons Attribution 4.0 International license (CC BY 4.0) which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. The remainder of the manuscripts are under different stages of submission for publication.

Chapter 1: General Introduction

Throughout the lifespan an organism will encounter countless stressors of varying degrees, duration and quality. Exposure to stress is an important precipitating factor in the development and maintenance of numerous psychiatric disorders including anxiety and mood disorders, as well as psychotic disorders (Heim & Nemeroff, 1999; Kendler, Karkowski, & Prescott, 1999; van, Stefanis, & Myin-Germeys, 2008). In general, stress can be conceptualized as a disruption of homeostasis or a threat to an organism's well-being. Stressors can be further described based on the "type" of challenge presented to the organism. These include, physiological/systemic challenges (drug withdrawal states, severe illness, changes in cardiovascular, respiratory, immune or respiratory functions); psychogenic/psychological stressors (e.g. interpersonal conflict, loss of a loved one, bullying, unemployment); and neurogenic/physical stressors (e.g. restraint stress, electrical foot shock, temperature variation). Psychogenic and neurogenic stressors can encompass both actual and anticipated events and stimuli. Furthermore, through recruitment of higher order cortical processing areas, an organism can appraise a given situation or stimulus as stressful (Anisman & Matheson, 2005) which highlights how one situation (e.g. public speaking) may be stressful for one individual but not another. Regardless of the source, the disruption of homeostasis caused by a stressor results in the implementation of adaptive behavioral and physiological responses designed to regain homeostasis. Both the behavioral and physiological response will depend on organismic variables (e.g. species, age, genetic, previous life experience and stressor history), as well as stressor characteristics (e.g. type, chronicity, predictability, controllability, intensity).

The Autonomic and Neuroendocrine Response to Stress

The physiological response to stressor exposure or stress-related cues involves the activation of interlocking systems designed to assist the organism in maintaining physiological integrity (Karatsoreos & McEwen, 2013). These systems include the autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis. Information about a given stressor is gathered from all major sensory systems and relayed to the brain, which organises the neural and endocrine response. Stressor-related cues, which can be exteroceptive (e.g. seeing a weapon in an attacker's hand) and/or interoceptive (e.g. drop in blood glucose), inform that brain of actual or potential shifts in homeostasis.

An organism's immediate neural response to a stressor is mediated by the ANS through the sympathetic and parasympathetic arms. This system is associated with the classic fast-acting "fight or flight" response, which involves rapid activation of the sympathetic arm in order to prepare the organism to respond accordingly. Briefly, the brainstem receives information from sensory inputs regarding homeostatic changes (Herman, Figueiredo, Mueller, & Ulrich-Lai, 2003), activating a reflex arc between the adrenal medulla and preganglionic sympathetic neurons in the intermediolateral cell column in the spinal cord (Ulrich-Lai & Herman, 2009). Activation of the reflex arc results in an increase in circulating noradrenaline and adrenaline levels, as well as diffuse actions on most parts of the body. These include changes within the body to prepare the organism to "fight or flee", such as energy mobilization, increased heart rate, peripheral vasoconstriction, and pupil dilation (Kandel, Schwartz, Jessell, Seigelbaum, & Hudspeth, 2013). The parasympathetic arm plays a complimentary role by controlling the duration of the stress response, and returning the body to a pre-stressor resting state (Kandel et al., 2013).

The HPA axis, the slower-acting endocrine response to a stressor, is also activated by signals of homeostatic imbalance via specific brain sites in the brainstem and certain forebrain limbic structures (Karatsoreos & McEwen, 2013). Excitatory limbic structures convey stressor-related information by activating cells in the paraventricular nucleus of the hypothalamus (PVN). The PVN is responsible for the production and release of corticotrophin-releasing factor (CRF), a key regulatory hormone in the stress response (Kandel et al., 2013). Cells in the PVN release CRF and arginine vasopressin (AVP) at the median eminence, which serve as an interface between the neural and peripheral endocrine systems (Yin & Gore, 2010). Release at the median eminence allows CRF and AVP to travel to the anterior portion of pituitary gland through the portal capillary system. CRF stimulates the corticotropic cells in the anterior pituitary to synthesize and release adrenocorticotropic hormone (ACTH), which enters the systemic blood stream. ACTH travels to the adrenal cortex to stimulate the release of glucocorticoids (cortisol in humans and corticosterone in rodents). Glucocorticoids assist in the mobilization of energy stores in order to provide the brain, skeletal muscles and cardiac muscles with the resources required to respond to a stressor.

The activity of the HPA axis is regulated by a negative feedback loop at the level of the pituitary, and hypothalamus whereby rising levels of glucocorticoids inhibit further CRF and ACTH release (Checkley, 1996; Herman et al., 2003; Ulrich-Lai & Herman, 2009). Inhibition of HPA axis activity is thought to occur through a fast acting as well as delayed-feedback mechanisms. The fast-acting system is proposed to involve non-genomic effects; however the mechanism of action and the specific receptor involvement is unclear at this time (Bamberger, Schulte, & Chrousos, 1996; de Kloet, Rots, & Cools, 1996; Keller-Wood & Dallman, 1984; Ulrich-Lai & Herman, 2009). The delayed feedback system involves genomic alterations

following the binding of two types of receptors; glucocorticoid (GR) and mineralocorticoid receptors (MR) (Checkley, 1996; Herman et al., 2003; Smith & Vale, 2006; Ulrich-Lai & Herman, 2009). While GRs have a low affinity for glucocorticoids, they extensively bind when relatively high concentrations of glucocorticoids are present (Reul & de Kloet, 1985). MRs have a higher affinity for glucocorticoids and are more occupied at periods of basal secretion (Reul & de Kloet, 1985; Reul & de Kloet, 1986). These observations suggest that GRs are involved in the mediation of the negative feedback loop, while MRs regulate basal HPA tone (Dallman et al., 1989; de Kloet et al., 1996; Ratka, Sutanto, Bloemers, & de Kloet, 1989; Smith & Vale, 2006). GRs are widely expressed in both peripheral and brain tissues; however two key regions appear to be implicated in negative feedback loop; the hypothalamus and the hippocampus. Local infusion of glucocorticoids at the hypothalamus reduces excitatory neural activity at the PVN, where high levels of GRs are expressed (Di, Malcher-Lopes, Halmos, & Tasker, 2003; Sawchenko, 1987; Smith & Vale, 2006). Likewise, the hippocampus also contains a high concentration of both GRs and MRs (Herman et al., 2003; Jacobson & Sapolsky, 1991) and administration of glucocorticoids or hippocampal stimulation results in a reduction in glucocorticoid release (Diorio, Viau, & Meaney, 1993; Dunn & Whitener, 1986; Furay, Bruestle, & Herman, 2008; Jacobson & Sapolsky, 1991; Rubin, Mandell, & Crandall, 1966).

Mediation of HPA axis activity through the aforementioned negative feedback loop is an important physiological adaption, as protracted activation of this system can be maladaptive and detrimental to an organism. However, this feedback system is best equipped to handle short-term stressors and chronic stress appears to overloads its functionality. Indeed HPA axis dysregulation has been implicated in the development of in a number of stressor-related disorders, including depression and anxiety disorders (Lopez-Duran, Kovacs, & George, 2009). Altered functionality

and structural changes to brain regions involved in the control of HPA axis activity have been documented following exposure to chronic stress (Ulrich-Lai & Herman, 2009). For example, exposure to a chronic stress regime has been shown to facilitate HPA axis responses to novel stressors (Akana et al., 1992; Bhatnagar & Dallman, 1999; Ulrich-Lai et al., 2007), as well as dampen the negative feedback system (Mizoguchi, Ishige, Aburada, & Tabira, 2003; Reul & de Kloet, 1985; Ulrich-Lai & Herman, 2009). The impact of chronic stress on HPA axis functionality and structure will be discussed in more detail in the context of specific stress regimes described in this introduction.

The Behavioral Response to Stress and Stress-Related Paradigms

Stressors also evoke an overt behavioral response, which is in part influenced by the internal neurophysiological, neuroendocrine and neurochemical changes that accompany stressor exposure. As mentioned previously, exposure to stress has been implicated in the pathogenesis of anxiety and depressive disorders (Heim & Nemeroff, 1999; Kendler, Karkowski, & Prescott, 1999; van, Stefanis, & Myin-Germeys, 2008). Animal models have played a pivotal role in the investigation of the neurobiological mechanisms underlying psychiatric disorders and their treatments. While animal models are unable to fully encompass all aspects of psychiatric illness, models have been developed to characterize certain aspects or symptoms of a given disorder (Anisman & Matheson, 2005). In rodents, markers of stress and/or anxiety include changes in food intake, increases in grooming or scratching, decreased exploration of a novel environment and decreased social behaviors (Campos, Fogaca, Aguiar, & Guimaraes, 2013; Ferre et al., 1995; File & Seth, 2003; Itoh, Takashima, Itoh, & Morimoto, 1994; Krahn, Gosnell, Grace, & Levine, 1986; Rodgers et al., 1999; Spruijt, Van Hooff, & Gispen, 1992). Furthermore, the availability of well-established behavioral paradigms allows the investigation of anxiety-like and depressive-

like behaviors following a given experimental manipulation (e.g. drug treatment, stress exposure). The following section will briefly describe selected behavioral paradigms used to evaluate anxiety- and depressive-like behaviors, as well as commonly used neurogenic and psychogenic models of stress. A description of relevant neuroendocrine and neurochemical consequences of stress models will also be discussed. While gender has been shown to impact vulnerability to stressors, as well as the development of stress-related pathologies (Anisman & Merali, 1999), gender differences are not the focus of the present review; thus the majority of information presented will detail studies involving male rodents.

Test of anxiety- and depressive-like behavior. Numerous behavioral paradigms have been developed to investigate various aspects of depressive- and anxiety-like behaviors in rodents. Included are a number of paradigms based on ethological situations (e.g. fear of open spaces in open field test) as well as tests that require conditioning (e.g. tone paired with foot shock in conditioned emotional response testing). A number of review articles are available on behavioral tests of anxiety- (Campos et al., 2013; File & Seth, 2003; Kumar, Bhat, & Kumar, 2013) and depressive-like behaviors (Abelaira, Reus, & Quevedo, 2013). The present sections will focus on the open field test (OF), the elevated plus maze (EPM), the social interaction test (SI), and the forced swim test (FST).

The OF test, EPM and SI test are all considered to be unconditioned models of anxiety, and are based on ethological constructs (Kumar et al., 2013). The EPM is probably the most widely used behavioral test for anxiety-like behavior, which is based on a conflict between an animal's desire to explore novel environments (including food foraging) and an innate fear of elevated and unprotected spaces (where the rodent is vulnerable to predators; (Campos et al., 2013; Kalueff & Tuohimaa, 2004). Anxiety-like behavior in the EPM is inferred from decreases in the total time

spent exploring and number of entries into the open arm(s) of the maze, as well as increases in the time spent in the closed arms of the maze (Carobrez & Bertoglio, 2005; Griebel, Rodgers, Perrault, & Sanger, 1997; Kalueff & Tuohimaa, 2004). Based on a similar ethological principle, the OF test is another commonly used paradigm to assess anxiety-related behavior. This test is thought to measure general emotionality, as well as exploratory behavior (Asano, 1986; Eilam, 2003; Kalueff & Tuohimaa, 2004; Walsh & Cummins, 1976). In this test, a test animal is placed in the center of a large square arena which has been divided up into a central and peripheral area using marked out squares. An increase in anxiety-like behavior is inferred from an increase in total time spent in the peripheral zone of the maze (Kalueff & Tuohimaa, 2004; Simon, Dupuis, & Costentin, 1994; Treit & Fundytus, 1988). The SI test was the first anxiety-related behavioral test based on ethological concepts (File & Seth, 2003). A pair of rodents are placed in an arena and allowed to freely interact for a set time period. Decreases in social behaviors (e.g. grooming, following, play fighting, sniffing) with no concurrent changes in locomotion are indicative of an anxiogenic-like behavior (File & Seth, 2003).

The FST is a behavioral test that is widely used in the validation of anti-depressant agents. This test is based on learned helplessness (Porsolt, Anton, Blavet, & Jalfre, 1978; Porsolt, Le Pichon, & Jalfre, 1977), whereby an animal is placed in a cylindrical container filled with water from which they cannot escape. The test consists of an initial 15 min pre-test followed by a test session 24 hours later. The initial test session aims to teach the animal that no escape is possible and promotes behavioral despair (measured by a reduction in escape-directed behavior) (Bogdanova, Kanekar, D'Anci, & Renshaw, 2013; Porsolt et al., 1978). Antidepressant treatments reduce the amount of time engaged in passive-coping strategies (e.g. immobility) and increase escape-directed behavior (Cryan, Valentino, & Lucki, 2005). It is noteworthy that the

FST does not directly evaluate the existence of depressive-like symptomatology, but rather the effectiveness of a given compound or agent in reducing passive immobile behavior. Other behavioral measures related to depressive phenotypes include the tail suspension test in mice, and the sucrose preference test.

Models of social stress in rodents. Social stress is an inherent part of being human. Whether it is interpersonal conflict, meeting new people or our relative "position" within society, it is likely we face some type and degree of social stress on a daily basis. In fact, social stress may represent humanity's most important stressor and may play a role in the development of future psychopathology (Tamashiro, Nguyen, & Sakai, 2005). Traditionally, physical or neurogenic stressors have been the focus of modelling stress in animals. However, such stressors fail to capture the challenges and stressors that an animal typically encounters in their natural habitats. Furthermore, the physiological response to neurogenic and psychogenic stressors differ (Herman & Cullinan, 1997; Koolhaas, De Boer, De Rutter, Meerlo, & Sgoifo, 1997; Martinez, Phillips, & Herbert, 1998), which raises the question of whether or not neurogenic stressors fully capture the etiology of social-stress-induced psychopathology. As a result, greater attention has been focused on the development of laboratory models of stress that are more a kin to what an animal may face in their natural environment in hopes of better modeling social stress. In an animal's natural habitat it is common for a social stressor to take the form of competition over limited resources, such as nutrients, space and reproductive partners. Such competitions naturally lead to the formation of social hierarchies where one's place within the hierarchy dictates the level of access to resources. Thus social stressors in animals typically involve a varying degree of exposure to a conspecific animal (Blanchard, McKittrick, & Blanchard, 2001). In rodents, the

two most widely research models are the visible burrow system (VBS) and the resident intruder or social defeat model.

Visible burrow system. The VBS involves group housing mixed-gender rodents (four male and two female) continuously, for at least 14 days, in a colony environment involving tunnels and burrows (Blanchard et al., 1995). The VBS is proposed to better approximate a rodent's natural environment and allows for the development of dominance hierarchies amongst the males, which typically involve one dominant male, and three to four subordinates (Tamashiro et al., 2005). Not only does the VBS provide an opportunity to study stressors within a more naturalistic setting, the established hierarchies allow scientists to study the unique physiological and behavioral profiles of dominant and submissive animals. Furthermore, through manipulation of the colonies there is also the possibility to look at the impact of changes in social hierarchy (e.g. a dominant becoming submissive in a novel colony setting). Indeed the main focus of the VBS has been on characterizing the dominant and submissive phenotype, including neuroendocrine and metabolic profiles. Behaviorally, subordinate animals tend to show more defensive behaviors, and decreased reproductive (Blanchard et al., 1995; Blanchard & Blanchard, 1990; Tamashiro et al., 2004) and social behavior (Blanchard & Blanchard, 1989). While dominant animals display more offensive behaviors (Blanchard et al., 1995; Blanchard & Blanchard, 1990; Tamashiro et al., 2004) which may be associated with defending their status and also highlights that dominant animals do experience stress in the VBS as well (Blanchard, Sakai, McEwen, Weiss, & Blanchard, 1993; Tamashiro et al., 2004).

There are conflicting reports on the impact of the VBS on HPA axis functionality. Relative to rodents who are not housed in a complex social environment (e.g. standard housing conditions) basal plasma corticosterone levels are elevated in both subordinate (Blanchard et al.,

1995; De Goeij, Dijkstra, & Tilders, 1992; Ely, Caplea, Dunphy, & Smith, 1997; Ely & Henry, 1978; McKittrick, Blanchard, Blanchard, McEwen, & Sakai, 1995) and dominant (Blanchard et al., 1995; Blanchard et al., 1993; Dijkstra, Tilders, Hiehle, & Smelik, 1992; McKittrick et al., 2000; McKittrick et al., 1995) rodents. However, the reported differences between the dominant and subordinate profile varies within the literature. The majority of studies report elevations in plasma corticosterone amongst subordinate rodents relative to both controls and dominants (Blanchard et al., 1995; Blanchard et al., 1993; De Goeij et al., 1992; Ely et al., 1997; Ely & Henry, 1978; McKittrick et al., 1995). Similar findings have also been reported in primates (Sapolsky, 1982; Sapolsky, 1983; Shively, Laber-Laird, & Anton, 1997; Virgin, Jr. & Sapolsky, 1997). However, other studies involving primates have reported no differences or even lower levels of corticosterone (Gust, Gordon, Brodie, & McClure, 1996; McGuire, Brammer, & Raleigh, 1986; Mendoza, Coe, Lowe, & Levine, 1978) suggesting that the link between social status and HPA axis functioning may be more complex. Nevertheless, consistent reports of adrenal hypertrophy in both rodents (Barnett, 1958; Blanchard et al., 1995; McKittrick et al., 1995; Tamashiro et al., 2004) and primates (Shively & Kaplan, 1984; Uno, Tarara, Else, Suleman, & Sapolsky, 1989) suggest increased adrenal activity (Barnett, 1958) providing more evidence that the VBS is stressful. There is also some evidence to suggest that HPA axis responsivity to a novel stressor may be impacted by the VBS. Subordinate animals have been reported to display an equivalent or increased in HPA axis reactivity to a novel stressor compared to dominant animals (Ely & Henry, 1978; Sapolsky, 1983). However, some studies suggest an impaired HPA axis response to a novel stressor among subordinates (De Goeij et al., 1992; Virgin, Jr. & Sapolsky, 1997) and even a sub-set of subordinate animals who display long-term hypo-activation of the HPA axis response to a novel stressor (Albeck et al., 1997;

Blanchard et al., 1995; Blanchard et al., 2001; Tamashiro et al., 2004). In rodents, a subordinate position within the VBS has also been associated with altered metabolic functioning, including decreased body weight (Barnett, 1958; Barnett, Eaton, & McCallum, 1960; Blanchard et al., 1995; Dijkstra et al., 1992; Haller, Fuchs, Halasz, & Makara, 1999; Tamashiro et al., 2004), reductions in leptin and insulin (Tamashiro et al., 2004), and, following a recovery period, increases in visceral to subcutaneous fat ratio (Tamashiro et al., 2004). Despite varying results in the literature, the VBS does indeed appear to result in stress-induced changes in neuroendocrine and metabolic functioning, which may be dependent on one's position within the social hierarchy. Not only does this paradigm offer an ethologically relevant method of study social stressors, it also allows the investigation of naturally occurring individual differences in response to a stressful environment (e.g. subordinate vs. dominant stance).

Resident intruder model/social defeat. The resident-intruder model has also been proposed to be an ethologically relevant model to study the impact of social stress in rodents (Miczek, 1979; Rygula et al., 2005; Wood, Walker, Valentino, & Bhathnagar, 2010). The methodology of the resident intruder paradigm differs greatly in the literature. Main differences involve variations in chronicity, unpredictability, and habituation (Patterson & Abizaid, 2013). In this model, a rat (intruder) is introduced into the home cage of a larger, more aggressive rat (resident). The intruder rat is often subject to aggressive behaviors from the resident designed to demonstrate dominance and establish social hierarchy (Miczek, 1979). The typical result of these interactions involves the assumption of a submissive posture on the part of the intruder and this has been described as social defeat (Wood et al., 2010). Following defeat, the resident and intruder are typically separated within the resident's cage by a divider, which prevents further physical contact between the resident and intruder while maintaining olfactory and visual cues

and thus maintaining the psychosocial nature of the stress (Rygula et al., 2005). Adequate screening and pre-training of residents allows the experimenter to manipulate the formation of the social hierarchy by ensuring that the intruder rat will be defeated. As mentioned previously, different laboratories employ differing techniques, include those that briefly (e.g. 5 to 60 min) expose intruders to a different resident per day for a set number of days (Bhatnagar & Vining, 2003; Bhatnagar, Vining, Iyer, & Kinni, 2006; Chuang et al., 2011; Foster, Solomon, Huhman, & Bartness, 2006; Keeney et al., 2006; Lutter et al., 2008; Papciak, Popik, Fuchs, & Rygula, 2013; Patki, Solanki, Atrooz, Allam, & Salim, 2013; Razzoli, Carboni, Guidi, Gerrard, & Arban, 2007; Rygula et al., 2005; Wood et al., 2010), thus allowing the study of unpredictable social stress. In contrast, other methodologies expose the intruder to the same resident rat each day (Becker et al., 2008; Buwalda et al., 1999; Fekete et al., 2009; Hollis, Wang, Dietz, Gunjan, & Kabbaj, 2010; Panksepp, Burgdorf, Beinfeld, Kroes, & Moskal, 2007; Pulliam, Dawaghreh, Alema-Mensah, & Plotsky, 2010; Tidey & Miczek, 1996) or chronically house the intruder with the resident with the cage divider in place (Bartolomucci et al., 2001; Bartolomucci et al., 2004; Bartolomucci et al., 2010; Keeney et al., 2006; Moles et al., 2006; Patterson, Khazall, Mackay, Anisman, & Abizaid, 2013; Solomon, Foster, Bartness, & Huhman, 2007). Exposure to the same resident per day has been proposed to lead to eventual habituation due to stressor predictability (Patterson & Abizaid, 2013).

Numerous studies have investigated the impact of social defeat in male intruder rodents. In particular, social defeat has been associated with altered HPA axis activity, increased depressive-like behaviors, promotion of anhedonia, decreased social interaction, increases in the propensity to use psychostimulants, and increased risk for cardio-vascular disease and other health related pathologies (Becker et al., 2008; Buwalda et al., 1999; Chang et al., 2009; Covington, III et al.,

2005; Heinrichs, Pich, Miczek, Britton, & Koob, 1992; Miczek, Covington, III, Nikulina, Jr., & Hammer, 2004; Rygula et al., 2005; Von Frijtag et al., 2000; Wood et al., 2010; Wood, Baez, Bhatnagar, & Valentino, 2009). Behaviorally, the main focus in the social defeat literature has been on behavioral paradigms measuring depressive-like symptomatology. Regardless of the varying methodology, exposure to social defeat in rodents has consistently been associated with increased immobility in the FST (Becker et al., 2008; Hollis et al., 2010; Keeney et al., 2006; Patki et al., 2013; Rygula et al., 2005; Rygula et al., 2008; Wood et al., 2010), as well as a reduced latency to immobility (Hollis et al., 2010). Increases in immobility have been reported to persist more than 3 weeks post defeat and responsive or prevented by anti-depressant treatment (Berton, Durand, Aguerre, Mormede, & Chaouloff, 1999; Hollis et al., 2010; Rygula et al., 2005; Rygula et al., 2008). However, the reported effects of social defeat on indices of anhedonia, as measured by the sucrose preference test, are inconsistent. The majority of studies report a decreased preference for a sucrose solution among defeated rats (Becker et al., 2008; Keeney et al., 2006; Krishnan et al., 2007; Patki et al., 2013; Rygula et al., 2005; Rygula et al., 2008); however, others have reported no changes in sucrose preference following defeat (Hollis et al., 2010; Meerlo, Overkamp, Daan, Van Den Hoofdakker, & Koolhaas, 1996). One study investigating varying concentrations of sucrose solutions reported no differences in sucrose preference (Hollis et al., 2010). The authors proposed that the absence of anhedonia may be due to the short-term nature of their defeat paradigm, suggesting that chronicity may be an important factor in the development of anhedonia. There is some evidence to suggest that exposure to social defeat promotes the development of anxiety-like behavior, as defeated rats have been reported to show decreased activity in the OF (Meerlo, Overkamp, Benning, Koolhaas, & Van Den Hoofdakker, 1996; Meerlo et al., 1996; Patki et al., 2013; Rygula et al., 2005), as well as

spend less time in the open arms of the EPM (Berton et al., 1999; Patki et al., 2013). Furthermore, social avoidance/decreased social contact following defeat has been reported in both mice (Berton et al., 2006; Krishnan et al., 2007; Meerlo et al., 1996; Tsankova et al., 2006) and rats (Hollis et al., 2010; Kudryavtseva, 2003; Razzoli et al., 2007). Increased contextual fear persisting 4-6 weeks post defeat has also been reported (Hollis et al., 2010).

Social defeat has also been shown to impact HPA axis activity. In male rats, a single episode of social defeat results in an increase in circulating corticosterone (Koolhaas et al., 1997), not-surprisingly demonstrating the ability of social defeat to activate the HPA axis. Longer term consequences lasting days to weeks have been documented following one or two social defeat exposures, including alterations in MR and GR binding in the hippocampus (Buwalda et al., 1999; Ruis et al., 1999), increased concentrations of CRF messenger ribonucleic acid (mRNA) in the PVN (Keeney et al., 2006), as well as impairments in dexamethasone suppression of HPA axis reactivity (Buwalda et al., 1999). Impairments in HPA axis negative feedback have also been reported to last for several weeks following defeat (Buwalda et al., 1999; Ruis et al., 1999). Increases in both ACTH and corticosterone, which persists as long as 24 days following acute defeat (3 day protocol), have been observed when rats are re-exposed to contextual cues associated with defeat (Razzoli et al., 2007). Furthermore exposure to defeat does evoke a stronger HPA axis response relative to novel cage placement (Bhatnagar et al., 2006). Studies investigating more chronic defeat exposure (e.g. 7 days and up) have demonstrated elevations in basal plasma corticosterone (Berton, Aguerre, Sarrieau, Mormede, & Chaouloff, 1998; Haller et al., 1999; Patki et al., 2013), as well as increased adrenal weight (Berton et al., 1998; Haller et al., 1999). While one day of social defeat followed by six days of threatened defeat (e.g. place in resident's cage with wire mesh divider in place) does not appear

to impact basal corticosterone and adrenal weight (Bhatnagar & Vining, 2003) suggesting that the threat of defeat may not be sufficient to cause longer-term changes in HPA axis functionality. Nevertheless, an episode of defeat followed by threat has been shown to facilitate HPA axis response to a novel stressor (Bhatnagar & Vining, 2003).

As with the VBS, the behavioral and physiological profile of the intruder animal appears to be influenced by individual differences in the response to the resident. More recently, Wood and colleagues (2010) investigated the profiles of intruder rats with varying latencies to defeat. By separating rats into two phenotypes, short- vs long-latency to defeat, they characterised two distinct behavioral and endocrine profiles. In particular, a short-latency to defeat was associated with adrenal hypertrophy, hypersecretion of CRF, down-regulation of pituitary CRF_1 receptors and increased immobility in the FST. Whereas, a longer latency to defeat was associated with a more active coping response, which the authors suggest protected them from the neuroendocrine and behavioral consequences of defeat. Interestingly, intruder animals that that are more resistant and fight back during resident/intruder interactions are also reported to show less severe behavioral and physiological consequences (Meerlo, Sgoifo, De Boer, & Koolhaas, 1999). The impact of dominant and resilient status in the social defeat paradigm and its resulting consequences in mice has been extensively characterised by Bartolomucci and colleagues (Bartolomucci et al., 2004; Bartolomucci et al., 2005; Bartolomucci et al., 2009; Moles et al., 2006).

Social isolation. In contrast to the above mentioned stress paradigms, which are based on interaction among rodents, social isolation (e.g. single housing) also represents a form of social stress. Social isolation was proposed as a form of stress in the 1960s following reports that socially isolated rats displayed hyper-emotionality and sensitivity to handling (Hatch, Wiberg,

Balazs, & Grice, 1963; Hatch et al., 1965). There are numerous examples within the literature suggesting that social isolation promotes the development of anxiety-like behavior (Hall, Huang, Fong, & Pert, 1998; Hall, Huang, Fong, Pert, & Linnoila, 1998; Hall, Huang, Fong, Sundstrom, & Pert, 2000; Hellemans, Benge, & Olmstead, 2004; Holson, Scallet, Ali, & Turner, 1991; Jankowska, Pucilowski, & Kostowski, 1991; Lodge & Lawrence, 2003; Maisonnette, Morato, & Brandao, 1993; Parker & Morinan, 1986; Weiss, Pryce, Jongen-Relo, Nanz-Bahr, & Feldon, 2004; Wright, Upton, & Marsden, 1991). However the timing of social isolation appears to be important and it has been proposed that this form of social stress is most potent during early development (Einon & Morgan, 1977). As such, social isolation will be addressed in more detail forthcoming sections describing the impact of stress during the juvenile or adolescent period.

Models of non-social stressors in rodents. Despite the growing evidence in the human that social stressors are an important factor in the development of psychopathology, a large number of animal studies involve the application of a physical stressor. Physical stressors are either used on their own (once for an acute stress or repeated to add an element of chronicity), or used in conjunction with other forms of stressors in a chronic stress model(s). The most commonly used physical stressors include: restraint/immobilization, tail pinch/foot shock, cold stress and the chronic mild stress procedure, which can also involve social stressors. Due to the scope of the present review, only restraint/immobilization and chronic mild stress will be reviewed.

Restraint/immobilization. Restraint and immobilization stressors have been widely used as physical stressors in animal research. It is noteworthy, that while restraint/immobilization typically involves the application of a physical apparatus (e.g. restraint bag), this stressor is also proposed to have a psychological component (Buynitsky & Mostofsky, 2009). In rats, restraint

stressors typically involve confinement in some type of device designed to prevent and/or limit movement. Immobilization stressors are generally considered to be more severe and involve techniques to ensure no movement what-so-ever, which can be achieved by pinning the animal's limbs down to a board or by the injection of a temporary paralytic agent. Within the literature on restraint/immobilization stress there is a lot of variability in the methodology deployed, including restraint procedures (e.g. type of device used), frequency of exposure (one day vs. repeated exposure over multiple days), duration (e.g. 5 min to hours) and intensity (e.g. extent of immobilization). Furthermore, it has been suggested that a thorough review of the impact of restraint stressors are complicated by the fact that the word "restraint" is typically not included in the title, abstract or keywords of a given publication, making literature review challenging (Buynitsky & Mostofsky, 2009). The huge variety of methodologies as well as some incomplete descriptions of procedures used makes it difficult to compare the impact of restraint stress across studies. Nonetheless, regardless of the methodology employed, exposure to some form of restraint has been associated with increases in ACTH (Foster et al., 2009; Hauger, Lorang, Irwin, & Aguilera, 1990; Hauger, Millan, Lorang, Harwood, & Aguilera, 1988; Pecoraro, Reyes, Gomez, Bhargava, & Dallman, 2004), corticosterone (Gameiro et al., 2006; Hauger et al., 1988; Inoue et al., 1993; Lucas, Wang, McCall, & McEwen, 2007; Marissal-Arvy et al., 2007; Pecoraro et al., 2004) and CRF (Foster et al., 2009; Hauger et al., 1990; Inoue, Koyama, Muraki, & Yamashita, 1993; Kang, Cirrito, Dong, Csernansky, & Holtzman, 2007), increased anxiety-like behavior (Gameiro et al., 2006; Heinrichs et al., 1994; Mendonca & Guimaraes, 1998; Padovan & Guimaraes, 2000; Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002), decreased exploration in the open field (Conrad, LeDoux, Magarinos, & McEwen, 1999; Gameiro et al., 2006), decreased rate of weight gain (Krahn, Gosnell, & Majchrzak, 1990; Laconi et al., 2000;

McLaughlin, Gomez, Baran, & Conrad, 2007; Vyas et al., 2002), decreased performance in memory-based tests (Conrad et al., 1999; McLaughlin, Gomez, Baran, & Conrad, 2007), and increased response to acoustic startle (Khan & Liberzon, 2004). Exposure to repeated restraint results in the habituation of the ACTH and corticosterone responses, as well as CRF responses (Dallman, 2007; Ma, Lightman, & Aguilera, 1999). Interestingly, HPA axis response to heterotypic stressors is preserved or even sensitized after repeated restraint (Aguilera, 1998; Aguilera, Pham, & Rabadan-Diehl, 1994; Ma et al., 1999; Marti, Gavalda, Gomez, & Armario, 1994). Additional behavioral, physiological and morphological impacts of restraint stress have been extensively reviewed (Buynitsky & Mostofsky, 2009; Glavin, Pare, Sandbak, Bakke, & Murison, 1994).

Chronic mild stress procedure. The chronic mild stress (CMS) procedure was developed by Willner and colleagues (Willner, Muscat, & Papp, 1992b; Willner, Towell, Sampson, Sophokleous, & Muscat, 1987) based on clinical and preclinical data regarding the role of chronic stressors in the development of depression (Katz, 1982). Also referred to as chronic unpredictable, variable, or intermittent stress, CMS involves the repeated application of mild stressors over a prolonged period of time (anywhere from 10 days to 8 weeks). Stressors are presented in an unpredictable fashion based on random selection from a list of potential stressors. Examples include restraint, water or food deprivation, inclination of homes cages, damp bedding, predator odour, overcrowded housing, tail pinch, alterations to the light/dark cycle, loud noises and so on (Duncko, Kiss, Skultetyova, Rusnak, & Jezova, 2001; Muscat & Willner, 1992; Ostrander, Ulrich-Lai, Choi, Richtand, & Herman, 2006; Willner, 1991; Willner, 1997; Willner et al., 1992b). There is a considerable amount of evidence for the CMS procedure's ability to produce depressive-like profile in rodents, as well as mimic other physical, behavioral and

physiological changes associated with depression (Hill, Hellemans, Verma, Gorzalka, & Weinberg, 2012; Willner, 1991; Willner, 1997; Willner, 2005). Most notably, exposure to CMS has been associated with measures of anhedonia, including decreased preference for and intake of sucrose (Benelli, Filaferro, Bertolini, & Genedani, 1999; D'Aquila, Brain, & Willner, 1994; De & Schreiber, 1997; Duncko, Brtko, Kvetnansky, & Jezova, 2001; Duncko et al., 2001; Grippo, Beltz, & Johnson, 2003; Grippo, Moffitt, & Johnson, 2002; Grippo, Na, Johnson, Beltz, & Johnson, 2004; Harro et al., 1999; Kioukia, Bekris, Antoniou, Papadopoulou-Daifoti, & Christofidis, 2000; Kompagne et al., 2008; Konkle et al., 2003; Kopp, Vogel, Rettori, Delagrange, & Misslin, 1999; Muscat, Towell, & Willner, 1988; Papp, Willner, & Muscat, 1991; Sampson, Willner, & Muscat, 1991; Schweizer, Henniger, & Sillaber, 2009; Willner et al., 1987; Zurita, Martijena, Cuadra, Brandao, & Molina, 2000) and saccharin solutions (Ayensu et al., 1995; Harris, Zhou, Youngblood, Smagin, & Ryan, 1997; Hatcher, Bell, Reed, & Hagan, 1997; Katz, 1982; Pucilowski, Overstreet, Rezvani, & Janowsky, 1993; Willner et al., 1987). While there have been some criticisms regarding the proposed anhedonic effects of CMS (Matthews, Forbes, & Reid, 1995), accumulating evidence suggests that the observed decreases in sucrose (or saccharin) solutions are related to alterations in the hedonic value of these solutions as opposed to reductions in body weight and food consumption (Gronli et al., 2004; Willner, Moreau, Nielsen, Papp, & Sluzewska, 1996). Furthermore, CMS has been associated with deficits in other measures of hedonic reactivity, including intake of a palatable food (Di, Loddo, & Tanda, 1999; Ducottet & Belzung, 2004; Gamaro, Manoli, Torres, Silveira, & Dalmaz, 2003; Gamaro, Prediger, Lopes, & Dalmaz, 2003; Griffiths, Shanks, & Anisman, 1992; Sampson, Muscat, Phillips, & Willner, 1992), conditioned place preference to food (Benelli et al., 1999; D'Aquila, Monleon, Borsini, Brain, & Willner, 1997) and drugs of abuse (Muscat, Papp, &

Willner, 1992; Papp, Lappas, Muscat, & Willner, 1992; Papp et al., 1991; Valverde, Smadja, Roques, & Maldonado, 1997), as well as brain stimulation reward (Moreau, Bourson, Jenck, Martin, & Mortas, 1994; Moreau, Jenck, Martin, Mortas, & Haefely, 1993; Moreau, Scherschlicht, Jenck, & Martin, 1995; Nielsen, Arnt, & Sanchez, 2000) and dopamine release (Di et al., 1999; Di & Tanda, 1997). Observed decreases in sucrose and saccharin intake have also been reported to be responsive to antidepressant treatment (Benelli et al., 1999; Bertrand, Smadja, Mauborgne, Roques, & Dauge, 1997; Katz, 1982; Kim et al., 2005; Kioukia et al., 2000; Luo & Tan, 2001; Marona-Lewicka & Nichols, 1997; Muscat et al., 1988; Papp, Moryl, & Willner, 1996; Sampson et al., 1991; Willner et al., 1987; Zhang et al., 2004). Indeed, other behavioral markers of depressive-like symptoms have also been reported following CMS including increased immobility in the FST (Bielajew et al., 2003; Garcia-Marquez & Armario, 1987; Griebel et al., 2002a; Griebel et al., 2002b; Griebel, Stemmelin, & Scatton, 2005; Kompagne et al., 2008; Molina, Heyser, & Spear, 1994; Papp et al., 1992; Sikiric et al., 2000; Strekalova, Spanagel, Bartsch, Henn, & Gass, 2004), learned helplessness (Gambarana et al., 1999a; Gambarana et al., 1999b; Gambarana, Scheggi, Tagliamonte, Tolu, & De Montis, 2001; Murua, Gomez, Andrea, & Molina, 1991; Secoli & Teixeira, 1998), decreased sexual behavior (Brotto, Gorzalka, & LaMarre, 2001; D'Aquila et al., 1994; Gronli et al., 2005) and aggression (D'Aquila et al., 1994; Gambarana et al., 2001; Ossowska, Danilczuk, Klenk-Majewska, Czajkowski, & Zebrowska-Lupina, 2004; Pardon, Gerardin, Joubert, Perez-Diaz, & Cohen-Salmon, 2000) and reduced self-care (Alonso et al., 2004; Ducottet & Belzung, 2004; Ducottet, Griebel, & Belzung, 2003; Griebel et al., 2002a; Griebel et al., 2002b; Mineur, Prasol, Belzung, & Crusio, 2003; Pardon et al., 2000). While the impact of CMS on depressive-like behaviors appears to be generally consistent, the effects of CMS on anxiety-like behavior are more suspect.

Studies to date would seem to suggest that CMS does not engender long-term anxiety as measured by the EPM (D'Aquila et al., 1994; Kompagne et al., 2008; Li, Zheng, Liang, & Peng, 2010; Mineur, Belzung, & Crusio, 2006) and light/dark box (Ducottet & Belzung, 2004; Ducottet et al., 2003; Kopp et al., 1999; Rossler, Joubert, & Chapouthier, 2000; Schweizer et al., 2009). There have been some reports of reduced social behaviors following CMS (D'Aquila et al., 1994; Kompagne et al., 2008). In general the behavior of CMS-stressed animals in behavioral tests of anxiety is more consistent with an anxiolytic profile, suggesting that the CMS may not induce an anxiety-like state.

The CMS procedure has also been associated with numerous neurobiological effects in a variety of neurochemical systems (refer to Hill et al. 2012 for an extensive review). While CMS has been shown to parallel multiple aspects of the neurochemical profiles associated with depression, the present review will focus on the impact of CMS on markers of HPA axis reactivity and MR and GR receptors. Reports on the effect of CMS on basal corticosterone are inconsistent. While the majority of studies report corticosterone hypersecretion following CMS (Ayensu et al., 1995; Bielajew, Konkle, & Merali, 2002; Froger et al., 2004; Grippo et al., 2005; Grippo, Francis, Beltz, Felder, & Johnson, 2005; Konkle et al., 2003; Patterson, Ducharme, Anisman, & Abizaid, 2010; Song, Che, Min-Wei, Murakami, & Matsumoto, 2006), some report no effects of CMS (Azpiroz et al., 1999; Duncko et al., 2001; Paternain et al., 2011; Silberman, Wald, & Genaro, 2002; Willner et al., 1987). Nevertheless, CMS has been associated with downregulation of GR mRNA expression (Froger et al., 2004; Kim et al., 1999; Mao, Ip, Ko, Tsai, & Che, 2009; Pan, Wang, Qiang, Zhang, & Kong, 2010; Xu et al., 2006; Yin et al., 2007; Zheng et al., 2006) and reduced GR cystolic binding in the whole hippocampus (Froger et al., 2004; Kim et al., 1999), as well as regionally specific reductions in the dentate gyrus (Herman,

Adams, & Prewitt, 1995; Yau, Noble, & Seckl, 2001) and CA1 region (Herman et al., 1995). These observations suggest that there may be some alterations in HPA axis feedback control (Froger et al., 2004), which would indeed be consistent with corticosterone hypersecretion. MR mRNA expression (Yin et al., 2007) and MR cystolic binding (Kim et al., 1999) is also reduced in the hippocampus following CMS. Collectively, the neurochemical and behavioral data suggest that CMS may be a useful model to study depression; however, the inconsistencies in the literature have led some researcher to conclude that this model may not be reliable (Nestler et al., 2002).

Stress in the Juvenile Period

A large majority of the stress literature in rodents involves the use of adult rats. While there are some studies focusing on the prenatal and neonatal periods (Holmes et al., 2005; Molet, Maras, Avishai-Eliner, & Baram, 2014; Veenema, 2009), the body of literature focusing on the adolescent or juvenile period is relatively sparse and more recent. In rodents the juvenile or adolescent period spans from weaning at post-natal day (PD) 21 to PD 59, and can be further subdivided into the early adolescence period (PD 21-34), mid-adolescence (PD 34-46) and late adolescence (PD 46-59) (McCormick & Mathews, 2010; Tirelli, Laviola, & Adriani, 2003). Pubertal onset is generally in mid-adolescence, and rats reach sexual maturity by PD 60 (McCormick & Mathews, 2010; Spear, 2000). To date, the literature has demonstrated that stressful life experiences are important factors in the development of anxiety and depressive disorders (Heim & Nemeroff, 1999; Kendler et al., 1999; van et al., 2008). Interestingly, the average age of onset for lifetime depressive and anxiety disorders is in adolescence (Kessler et al., 2005), which highlights the relevancy of research targeting this age group.

Juvenility as a critical window. Adolescence can be a stressful time and involves a number of novel and unexpected experiences, as well as developmental changes (Murray, Byrne, & Rieger, 2011). In addition to the bodily changes that accompany puberty, adolescence also involves a shift of focus towards peer and sexual relationships, as well as exposure to novel situations (Panksepp, 1981; Spear, 2000). It has been argued that the large majority of stressors encountered in adolescence include a social component (Buwalda, Geerdink, Vidal, & Koolhaas, 2011). These include the formation of new peer relationship, interpersonal conflicts, parental conflict, and peer victimization. Indeed, peer victimization is not uncommon, with approximately 10% of children being the victim of serious bullying (Juvonen, Graham, & Schuster, 2003). Among Canadian youth 1 in 3 adolescents report experiencing bullying (Molcho et al., 2009), and reports of occasional or frequent bullying during schools years are reported by 38% and 30% of adult Canadian men and women, respectively (Kim & Leventhal, 2008). While the challenges of adolescence offer youth opportunities for growth and development, they might also have ramification for the individual's ability to contend with later stressor experiences (Compas, Hinden, & Gerhardt, 1995). It has been suggested that this major biological transition period renders adolescents more sensitive to the effects of stressors and subsequently the development of stressor-related psychopathologies (Avital & Richter-Levin, 2005).

Indeed, the juvenile phase represents a critical period in development during which there is substantial cerebral development and reorganization as well as altered HPA axis function (Bingham, Gray, Sun, & Viau, 2011; McCormick & Mathews, 2010). Animal studies have shown unique effects of stress during adolescence on both behavior and HPA activity. In general, pre-pubertal rats exhibit higher or prolonged ACTH and corticosterone release compared to adults (McCormick & Mathews, 2010). However, these effects are sex dependent and vary depending on the stressor

employed (McCormick & Mathews, 2010). Prolonged stress induced activation of the HPA axis in adolescents has been proposed to be related to immaturity of the HPA negative feedback system (Spear, 2000); however the proposed immaturity of the negative feedback systems appears to be independent of glucocorticoid receptor expression, as GR and MR binding capacity does not appear to differ between adults and adolescents (Vazquez & Akil, 1993). Furthermore, there does not appear to be a difference in glucocorticoid-induced up-regulation or down-regulation of GR receptors in adolescence (Sapolsky, Meaney, & McEwen, 1985; Vazquez, 1998). Adolescence is further characterized as a unique stage in brain development, as regions related to emotional and learning processes, such as the prefrontal cortex, hippocampus, and the amygdala, all undergo substantial remodeling during this phase and have also been proposed to be exquisitely vulnerable to the effects of stress (Spear, 2000). For an extensive review of the neurobiological changes that accompany adolescence readers are referred to Spear, 2000.

Models of social stress in juvenility. As mentioned previously, adolescence in humans is marked by a shift towards peer groups and an increase in sociability. This increased social contact is proposed to assist in the development of social skills, as well as provide an outlet for positive experiences (Csikszentmihalyi, Larson, & Prescott, 1977). Interestingly, sociability in the adolescent time frame is also proposed to play an important role in rodents. Indeed, juvenile rodents display increased social interactions relative to adults, as well as increases in play behavior which is proposed to assist rodents in developing and practice the necessary social behaviors needed in adulthood (Einon & Morgan, 1977; Panksepp, 1981; van den Berg et al., 1999). In humans, concurrent with the increased time spent engaging with same-age peers is an increase in social conflicts (Laursen & Collins, 1994). Increased social contact, coupled with the adult literature on the impact of social stress models in adult rodents, highlights the importance

of investigating social stress during the adolescent period. In this regard, the social instability model, the resident intruder model and social isolation have been used as models of adolescent stress.

Social instability model. McCormick and colleagues have focused on characterizing the impact of chronic social stress in adolescents in both males and females using the social instability model. Based on a hypothesis of the moderating effects of social relationships on stress in rodents (Armario, Luna, & Balasch, 1983; Kiyokawa, Kikusui, Takeuchi, & Mori, 2004; Sachser, Durschlag, & Hirzel, 1998), this model involves a combination of limited daily social isolation (1 hour) followed by pair-housing with a novel cage mate from PD 30 to 45. Acute social isolation results in increased corticosterone release (McCormick et al., 2001; McCormick, Kehoe, Mallinson, Cecchi, & Frye, 2002), and subsequent housing with a novel cage mate results in a prolonged recovery period relative to isolated juvenile who are returned to a cage with a familiar partner (McCormick, Merrick, Secen, & Helmreich, 2007). Furthermore, adolescent rats in the social instability condition did not develop habituation to repeated isolation while rats returned to a familiar cage mate did (McCormick et al., 2007).

Exposure to social instability has been shown to have long-term implications for anxiety-in adulthood. When tested in adulthood, both male and female rats demonstrate anxiety-like behavior in the EPM and open field test following exposure to social instability between PD 30-45 (McCormick, Smith, & Mathews, 2008). In males this was characterized by an increased latency to enter the centre of the open field, increased latency to approach a novel object in the centre of the open field (Green, Barnes, & McCormick, 2013), as well as increased locomotor activity and less time spent in the open arms of the EPM (McCormick et al., 2008). Observed anxiety-like behavior in the open field was independent of locomotor activity, and no difference

in time spent exploring novel objects placed in the less anxiogenic periphery of the arena was observed (McCormick et al., 2012; McCormick, Nixon, Thomas, Lowie, & Dyck, 2010). Interestingly, this anxiety-like profile is only evident in adulthood, as rats tested immediately after the termination of social instability do not display anxiety-like behavior (McCormick et al., 2008). Increased anxiety-like behavior following termination of social instability stress has also been documented in mice (Schmidt et al., 2007; Schmidt et al., 2010; Sterlemann et al., 2008). In addition to increased anxiety-like behavior in the open field and EPM, male rats exposed to social instability stress also display altered social behavior. When tested in the social interaction test in adulthood, stressed rats displayed reduced social behaviors when paired with control rat and an unfamiliar rat that had also undergone social instability stress (Green et al., 2013). Social deficits in males also appears to extend to sexual behaviors, as rats exposed to social instability stress display reduced copulatory efficiency, reduced likelihood to complete an ejaculatory sequences and a longer latency to ejaculation (McCormick et al., 2013). Social instability stress does not appear to engender long-term depressive like behavior in rats. When tested in adulthood, no significant differences have been observed in time spent immobile in the FST among both male and female rats exposed to social instability stress (Mathews, Wilton, Styles, & McCormick, 2008); however, adult males were observed to spend more time climbing which the authors speculate may represent either an increased panic state or an active stress coping style (Mathews et al., 2008). Deficits in learning and memory have also been reported following social instability stress (Green & McCormick, 2013).

In juvenility, previous exposure to social instability stress does not alter basal corticosterone levels in adolescent male rats when sampled 24 hours after the termination of the stressor (Mathews et al., 2008; McCormick et al., 2007; McCormick et al., 2008); however, it did

potentiate the corticosterone response to a novel swim stressor (Mathews et al., 2008). These results are consistent with other reports of prolonged HPA axis responses in stressed adolescents (Cruz, DeLucia, & Planeta, 2008; Gomez, Houshyar, & Dallman, 2002; Romeo et al., 2006; Romeo, Karatsoreos, & McEwen, 2006; Romeo, Lee, Chhua, McPherson, & McEwen, 2004; Romeo, Lee, & McEwen, 2004; Vazquez & Akil, 1993). In contrast, when exposed to a more mild stress (e.g. confinement in the open arm of the EPM), stressed adolescent rats displayed a blunted corticosterone response, which the authors hypothesised may be related to the intensity of stressor or a result of brief inter-stress interval resulting in diminished adrenal capacity (McCormick et al., 2008). While social instability stress has been demonstrated to have a long-term impact on anxiety-like behavior in adulthood, this does not seem to translate to HPA axis functionality. Indeed, male previously exposed to social instability stress do not display prolonged HPA axis response to a novel stress 25 days after stressor termination (Mathews et al., 2008; McCormick, Robarts, Kopeikina, & Kelsey, 2005; McCormick et al., 2008).

Resident intruder model/social defeat. As with adult rodents, the resident intruder model has been used as a model of social subordination in adolescence. The methodology is similar to that of the adult model with the exception of using juvenile rats as intruders. Sexually mature adult rats are still used as resident rats. As in the adult literature, there is much variety in the methodology used in juvenile social defeat including: exposure during early adolescence (Bingham et al., 2011; Buwalda, Stubbendorff, Zickert, & Koolhaas, 2013); mid-adolescence (Bingham et al., 2011; Burke, Watt, & Forster, 2011; Iniguez et al., 2014; Novick, Miller, Forster, & Watt, 2013; Watt, Burke, Renner, & Forster, 2009); late adolescence (Coppens et al., 2011; Vidal et al., 2007; Vidal, Buwalda, & Koolhaas, 2011a; Vidal, Buwalda, & Koolhaas, 2011b); and exposure across the span of adolescence (Bingham et al., 2011; Bourke, Glasper, &

Neigh, 2014; Bourke & Neigh, 2011; Hayashida, Oka, Mera, & Tsuji, 2010; Kovalenko et al., 2014; Weathington, Arnold, & Cooke, 2012). The nature of defeat varies from chronic housing with an aggressive resident (Buwalda et al., 2013; Iniguez et al., 2014; Kovalenko et al., 2014), daily defeat sessions (Bingham et al., 2011; Bourke et al., 2014; Burke et al., 2011; Buwalda et al., 2013; Coppens et al., 2011; Hayashida et al., 2010; Novick et al., 2013; Vidal et al., 2011a; Watt et al., 2009; Weathington et al., 2012) and a combination of defeat and threat of defeat (Vidal et al., 2007; Vidal et al., 2011b). Studies also vary based on whether or not juvenile intruders are pair housed (Burke et al., 2011; Buwalda et al., 2013; Novick et al., 2013; Watt et al., 2009) or singly housed (Bingham et al., 2011; Bourke et al., 2014; Coppens et al., 2011; Hayashida et al., 2010; Vidal et al., 2007; Vidal et al., 2011a; Vidal et al., 2011b; Weathington et al., 2012) during the experiment.

The impact of social defeat appears to have varying effects based on age. Bingham and colleagues (2011) exposed different groups of Sprague-Dawley rats to 7 days of social defeat in early adolescence (PD 28-34), mid-adolescence (PD 42-48) and adulthood (PD 63-69). When tested 24 hours after the last defeat session, rats exposed to social defeat in early adolescence displayed increased defensive burying, as well as climbing in the FST (Bingham et al., 2011). The authors proposed that this behavioral profile may represent proactive coping (Bingham et al., 2011; Koolhaas et al., 1999). Interestingly, social defeat in early adolescence continued to impact behavior in adulthood; however, behavior shifted from a proactive stance with rats displaying decreased defensive burying when tested as adults. Rats defeated in adulthood also displayed reduced defensive burying. No immediate effects of defeat during mid-adolescence were observed (Bingham et al., 2011). Consistent with previous reports on the impact of social instability stress, early adolescent rats also displayed a prolonged corticosterone response to

defeat relative to adult and mid-adolescent rats. A significant elevation in basal corticosterone post juvenile defeat, which returns to baseline when sampled 2 days later, has been reported by others (Watt et al., 2009).

Another study evaluating the short-term impact of social defeat from PD 28-50 reported depressive-like behavior in the FST and sucrose preference test 5 days after the last defeat session (Bourke et al., 2014). Results in the FST appear to be in contrast to those reported by Bingham and colleagues (2011); however, it is noteworthy that the chronicity of defeat was much longer in this study, suggesting that chronic defeat may be associated with a different behavioral phenotype. Indeed, Hayashida and colleagues (2010) exposed rats to varying lengths of defeat protocol. Increased immobility in the forced swim test (24 hours post-defeat) was only evident in rats who had received 21 days of repeated social defeat (Hayashida et al., 2010) providing further support that chronic defeat exposure may be necessary for the development of a depressive phenotype. Increased immobility in the FST was still present when rats were allowed a week recovery between termination of defeat and FST testing. In mice, juveniles chronically housed with an aggressive resident also display increased depressive-like behavior in the FST and sucrose preference test, as well as increased anxiety-like behavior and social avoidance when tested 24 hours after the termination of housing conditions (Iniguez et al., 2014).

There is a growing body of literature on the long-term effects of juvenile social defeat on anxiety-like behavior in adulthood. Vidal and colleagues have demonstrated that social defeat and psychosocial stress (e.g. confinement in the resident cage when the resident is absent) between PD 45-58 can impact behavior in adulthood in Wistar rats, including increased anxiety-like behavior in the social approach task (Vidal et al., 2007; Vidal et al., 2011b). Exposure to two defeat sessions on PD 45 and 46 also results in anxiety-like behavior in the water conflict test

three weeks after defeat; however, increased anxiety is only evident when rats are tested in the defeat context indicating that the effects of social defeat did not generalize to other contexts (Vidal et al., 2011a). Other studies have reported no impact of juvenile social defeat on behavior in the social interaction test in male rats (Weathington et al., 2012).

Behavior in the EPM following juvenile social defeat appears to somewhat inconsistent. Some studies have reported no effects of social defeat on EPM behavior in adulthood (Bourke & Neigh, 2011; Buwalda et al., 2013; Hayashida et al., 2010), while others report either anxiogenic (Weathington et al., 2012) or anxiolytic profiles (Watt et al., 2009). Unfortunately, the large discrepancies in methodologies across these studies make it difficult to discern what could account for the observed variance in EPM behavioral outcome. However, it has been proposed that housing conditions may play an important role in the long-term impact of social defeat (Buwalda et al., 2011). Indeed, a combination of social defeat and psychosocial stress between PD 28-39 resulted in increased anxiety-like behavior in the EPM in rats tested during adulthood (PD 63-84) (Weathington et al., 2012). Rats in this study were singly-housed for the duration of this study. In contrast, Watt and colleagues (2009) reported a novelty-seeking profile in the EPM among doubly housed rats defeated during PD 35-40 when tested in adulthood. These rats displayed increased time in the open arm, as well as increased exploratory behavior in both the EPM and open field (Watt et al., 2009). While this provides some insight into the impact of housing conditions, the story does not appear to be clear-cut. Bourke and Neigh (2011) compared the impact of social defeat among singly-housed rats to doubly housed controls. In this study, social defeat did not impact behavior in the EPM among male rats; however among female rats a depressive-like profile in the FST and sucrose preference test were noted (Bourke & Neigh, 2011).

The impact of juvenile social defeat on HPA axis reactivity is relatively scarce. As mentioned previously, juvenile social defeat does induce short-term elevations in corticosterone, consistent with the hypothesis that in general, juvenile rats display prolonged corticosterone response to stressor relative to adults (Bingham et al., 2011; Iniguez et al., 2014; Watt et al., 2009). However no differences in basal corticosterone levels in adulthood have been reported between defeated rats and controls (Watt et al., 2009). Similarly, no difference in stressor-evoked corticosterone release following a swim stress in male rats has also been reported (Weathington et al., 2012). Nevertheless, there is some evidence to suggest that females may be more susceptible to long-term sensitization of HPA axis functioning following juvenile defeat (Weathington et al., 2012).

Social isolation. Social isolation/deprivation from weaning (typically PD21) results in a variety of long-lasting neurochemical and behavioral alterations in isolates relative to group-housed counterparts (Einon & Morgan, 1977; Heidbreder et al., 2000; Lapiz et al., 2003; Valzelli, 1973). Interestingly, these alterations are only evident when rodents are isolated during a critical window (PD 20 to 30) close to pubertal onset (Einon & Morgan, 1977; Robbins, Jones, & Wilkinson, 1996), as observed results of isolation in rat pups are not seen in adults when the same procedure is employed (Fone & Porkess, 2008). The procedure for isolation is relatively straightforward: rodent pups are housed individually from the first day of weaning from the dam. However, it is important that isolates are handled minimally as experimental manipulation of isolates (e.g. daily drug administration) has been shown to mitigate some of the long-term effects of isolation (Fone & Porkess, 2008). Relative lack of enrichment of the housing environment also appears to be germane as it impacts the outcome of isolation (Weiss, Feldon, & Domeney, 1999). The majority of studies isolate rodents for the duration of the study (Fone & Porkess,

2008); however, some studies involve re-socialization by returning isolates to a group housing condition in late adolescence or early adulthood (Lukkes, Vuong, Scholl, Oliver, & Forster, 2009; Lukkes, Mokin, Scholl, & Forster, 2009; Lukkes, Summers, Scholl, Renner, & Forster, 2009; van den Berg et al., 1999).

Social isolation during adolescence has been consistently linked with increased anxiety-like behavior in the EPM. For example, Long Evans rats isolated from PD 28 spent less time in the open arms, and made fewer open arm entries in the EPM when tested between PD 72-78 (Chappell, Carter, McCool, & Weiner, 2013). Similar results have been reported by Weiss and colleagues (2004) in Sprague Dawley rats. Increased anxiety-like behavior in the EPM was evident 13 weeks after social isolation beginning at PD 21 (Weiss et al., 2004). Increased anxiety-like behavior in adulthood has been reported by others (Hall, 1998; Hall et al., 1998; Hall et al., 2000; Hellemans et al., 2004; Jankowska et al., 1991; Lodge & Lawrence, 2003; Maisonnette et al., 1993; Parker & Morinan, 1986; Wright, Ismail, Upton, & Marsden, 1991; Wright et al., 1991). Interestingly, re-socialization does not appear to mitigate the effects of social isolation in the EPM. For example, Wright and colleagues (1991) isolated rats from PD 21-52 then re-socialized them for a period of 30 days. Despite re-socialization, isolates still displayed increased anxiety-like behavior in the EPM. Inconsistent reports of an anxiolytic profile or no effects of isolation on EPM behavior would suggest that the timing of isolation may be an important factor in the development of anxiety-like behavior. In this regard, Thorsell and colleagues (Thorsell, Slawecki, El, Mathe, & Ehlers, 2006) demonstrated an anxiolytic profile in both the EPM and open field when social isolation was initiated in later adolescence (PD 45). Similarly, no impact of social isolation on EPM behavior (tested on PD 62) was reported by among rats isolated from PD28-60 (Brenes, Padilla, & Fornaguera, 2009). These results are in

contrast to those reported by Chappell et al (2013); however, the duration of social isolation employed by Chappell et al (2013) was longer (44 vs. 32 days) suggesting that not only age of exposure but duration of isolation may also influence behavioral outcome in adulthood.

The impact of adolescent social isolation on social behavior also appears to be inconsistent. Studies investigating pre- to mid-adolescent social isolation followed by re-socialization has demonstrated reduced social approach behavior, as well as increased freezing and reduced social behavior in the social interaction test (Lukkes et al., 2009; Lukkes et al., 2009; van den Berg et al., 1999). However, an important caveat in these studies is the potential confounding effects of re-socialization. Indeed, studies involving chronic isolation with no re-socialization have reported that isolates display an increase in social interaction with novel conspecifics in adulthood (Ferdman, Murmu, Bock, Braun, & Leshem, 2007; Ikemoto & Panksepp, 1992; Niesink & Van Ree, 1982a; Niesink & Van Ree, 1982b; Rockman, Hall, Markert, & Glavin, 1987; Wongwitdecha & Marsden, 1996), suggesting that the re-socialization procedure may influence behavior in this paradigm. Depressive-like behaviors following adolescent social isolation are also inconsistent with some studies reporting impairments in the FST (Brenes, Rodriguez, & Fornaguera, 2008), and other reporting no impact (Hall et al., 1998; Hall, Sundstrom, Lerner, & Pert, 2001; Hong et al., 2012).

It is somewhat unclear as to whether or not social isolation impacts HPA axis functioning, as reported results are mixed. In general, most studies appear to report no impact of social isolation on basal corticosterone (Heidbreder et al., 2000; Lukkes et al., 2009; Scaccianoce et al., 2006; Toth, Mikics, Tulogdi, Aliczki, & Haller, 2011; Weiss et al., 2004) and ACTH levels (Schrijver, Bahr, Weiss, & Wurbel, 2002). No impact of social isolation on MR and GR expression in the hippocampus or adrenal weights has also been reported (Weiss et al., 2004).

Nevertheless, there have been some reports suggestive of increases in HPA axis tone including enhanced HPA response to acoustic startle (Weiss et al., 2004), as well as enhanced negative feedback following 10 min restraint stress (Lukkes et al., 2009). More recently, an enhanced HPA axis response to aggression was reported in Wistar rats isolated from PD 21 (Toth et al., 2011). However, impaired negative feedback has also been suggested, as exposure to social isolation was associated with an attenuation of dexamethasone-induced suppression of corticosterone release (Serra, Pisu, Floris, & Biggio, 2005).

Models of Non-Social Stress in Juvenility.

The majority of studies investigating the impact of non-social stressors in juvenility apply a combination of different stressors over a fixed period of time (3 to 14 days). The most commonly applied stressors appear to be elevated platform stress, swim stress, restraint, or foot shock. While these stressors are somewhat physical in nature, they are also proposed to have a psychological component. Generally, stressors are applied in early adolescence (i.e. before PD 34). In this regard, Richter-Levin and colleagues have focused on characterizing the long-term impact of exposure to a 3-day variable stress (swim stress, elevated platform, restraint) from PD 27-29. Exposure to this stress regime results in long-term anxiety-like behavior in both the open field (Horovitz, Tsoory, Hall, Jacobson-Pick, & Richter-Levin, 2012; Ilin & Richter-Levin, 2009) and EPM (Ilin & Richter-Levin, 2009; Jacobson-Pick & Richter-Levin, 2010; Jacobson-Pick & Richter-Levin, 2012), as well as impairments in avoidance learning (Horovitz, Tsoory, Yovell, & Richter-Levin, 2014; Ilin & Richter-Levin, 2009). Interestingly, the long-term anxiogenic effect of this juvenile stressor can be prevented by environmental enrichment (Ilin & Richter-Levin, 2009). Previous exposure to this paradigm also enhanced the behavioral response to stressor applied in adulthood (Jacobson-Pick & Richter-Levin, 2012). This enhanced

sensitivity to adult stressors has also been demonstrated after repeated exposure to platform stress (Avital & Richter-Levin, 2005; Jacobson-Pick, Elkobi, Vander, Rosenblum, & Richter-Levin, 2008). More recently, a modified version of Richter-Levin and colleagues (reduced severity of restraint stress) has also demonstrated long-term anxiety-like behavior in the EPM and social interaction test which was mitigated by access to a palatable diet (Mackay et al., 2014). Increased anxiety-like behavior on the EPM , reductions in exploratory behavior and impaired learning have also been reported follow exposure to a similar 3-day stress protocol (forced swim, elevated platform, foot shock) (Brydges, Hall, Nicolson, Holmes, & Hall, 2012; Luo et al., 2014; Saul et al., 2012; Tsoory, Cohen, & Richter-Levin, 2007; Tsoory & Richter-Levin, 2006), as well as 3 days of elevated platform stress (Avital, Ram, Maayan, Weizman, & Richter-Levin, 2006; Avital & Richter-Levin, 2005). The long-term implications of this paradigm on HPA axis functioning is less understood. Exposure to this 3-day stress paradigm has been associated with elevation in basal corticosterone (Ilin & Richter-Levin, 2009), or unaltered corticosterone (Jacobson-Pick & Richter-Levin, 2010; Mackay et al., 2014).

Protocols involving longer durations (seven days to four weeks) of stressors, like in the case of CMS paradigm in adult rats, have reported similar results to the aforementioned 3-day models. Notably, longer exposure to juvenile stress have been associated with impaired social behavior in adulthood (Oztan, Aydin, & Isgor, 2011; Poirier, Imamura, Zanoletti, & Sandi, 2014), an increased in anxiety-like behavior in the open field (Negron-Oyarzo, Perez, Terreros, Munoz, & Dagnino-Subiabre, 2014), EPM (Llorente et al., 2011; Wilkin, Waters, McCormick, & Menard, 2012) and light/dark box (Negron-Oyarzo et al., 2014), memory impairments (Llorente et al., 2011), as well as increased immobility in the FST (Oztan et al., 2011). However, one study investigating the impact of over thirty days of chronic variable stress (CVS) starting on PD 21

reported no long-term impact on locomotor behavior, sexual behavior and behaviors in the FST (Toth et al., 2008). Nonetheless, rats in this study displayed decreased basal evening corticosterone levels two weeks after the termination of CVS (Toth et al., 2008). Interestingly, some behavioral results appear to be age-dependent. For example, Wilkin and colleagues (2012) exposed Long-Evans rats to ten days of variable stressors during either early (PD 22-33) or late (PD 35-46) adolescents. Only rats exposed to stress during early adolescence displayed anxiety-like and depressive-like behaviors in adulthood, suggesting that rats may have been more sensitive to the behavioral impact of the stressor regime during early adolescence (Wilkin et al., 2012). While methodology appears to vary greatly across studies investigating the impact of physical stressors in juvenility, there appears to be ample evidence that stressors can have long-lasting implications on anxiety-like behavior in adulthood. Whereas the impact of juvenile physical stressors on depressive-like behavior and HPA axis appear to be less understood.

Coping with Stress

Although numerous definitions of coping exist, coping is generally referred to as conscious and willful actions an organism takes to mitigate or adapt to a challenge (Compas, Connor-Smith, Saltzman, Thomsen, & Wadsworth, 2001). It is noteworthy that the definition of coping does not encompass the efficiency of given strategy or action. In this regard, coping is differentiated from the concept of resilience. Where the former represents the action taken by an individual and the latter represents outcome of coping patterns have been applied in a competent and adaptive manner (Compas et al., 2001). Interestingly, the available options and limits of coping strategies are influenced by an individual's developmental age (Compas et al., 2001; Compas et al., 1995). Coping strategies can take many forms, such as talking to a friend, exercising, meditation, taking hot bath, leaving a stressful situation, avoiding conflict and so on.

Self-medication with food. A form of a coping strategy that of late has been receiving increasing attention is comfort eating. A stressor-induced preference for palatable foods (especially those with a high fat and/or sugar content) has been documented in both animals and humans (Dallman, 2010; Gibson, 2006; O'Connor, Jones, Conner, McMillan, & Ferguson, 2008; Zellner et al., 2006).This preference has been proposed to serve as a form of self-medication in order to protect against the effects of stress (Dallman, Pecoraro, & la Fleur, 2005). In humans, for instance, many women report stress eating, for which being overweight is a predictor (Warne, 2009). Higher levels of self-reported stress are associated with a greater desire to eat, including binge eating (Warne, 2009). Students who self-identify as stress-eaters have higher urinary cortisol during stressful periods, suggesting that the elevated cortisol may be associated with their increased desire to eat (Warne, 2009).

In animals, some studies have demonstrated that adult rats will increase their intake of a palatable food in response to a stressor (Bell et al., 2002; Dallman et al., 2006; la Fleur, Houshyar, Roy, & Dallman, 2005; Pecoraro et al., 2004), while other studies have failed to show an increase in palatable food preference in response to a stressor (Fachin et al., 2008; Kant & Bauman, 1993; Young, 2000). Access to a palatable food in adulthood has been proposed to protect against learned helplessness after an inescapable foot shock (Dess, 1992), reduce sympathetic responses to a stressor (Buwalda, Blom, Koolhaas, & van Dijk, 2001; Christiansen, Dekloet, Ulrich-Lai, & Herman, 2011; Fachin et al., 2008; Kant & Bauman, 1993; la Fleur et al., 2005; Pecoraro et al., 2004; Ulrich-Lai et al., 2007; Ulrich-Lai et al., 2010; Ulrich-Lai, Ostrander, & Herman, 2011; Young, 2000), and diminish hypothalamic-pituitary adrenal (HPA) axis activity (Foster et al., 2009; Levin, 1996; Strack, Akana, Horsley, & Dallman, 1997; Zeeni et al., 2012).

Stress, adolescence and coping with food. As mentioned previously, adolescence is a challenging period of development, accompanied by a wide variety of life experiences and changes. This includes exposure to a variety of stressors, stemming from interpersonal challenges to an increased level of responsibilities (Byrne, Davenport, & Mazanov, 2007; Moksnes, Byrne, Mazanov, & Espnes, 2010). Exposure to a variety of stressful life events, as well as daily stressors, is associated with clinically significant psychological symptoms among adolescents (e.g. depression, anxiety; (Byrne et al., 2007; Charbonneau, Mezulis, & Hyde, 2009; Moksnes et al., 2010). Recent survey data collected by the American Psychological Association suggests that stress in this age group may be on the rise. According to survey results, thirty-one percent of adolescents (1,018 respondents between 13 to 17 years of age) reported feeling overwhelmed by stress (American Psychological Association, 2014). Similar numbers of teens (31 percent) reported that they perceived their levels of stress to have increased within the past year and approximately thirty-four percent of adolescents envisioned their stress level continuing to increase over the coming year. In contrast, only sixteen percent of teens felt that their level of stress had decrease over the past year. Most alarmingly, nearly half of respondents (42 percent) reported that they were not taking the appropriate steps to managing their stress (American Psychological Association, 2014). Collectively, these results highlight the growing importance of investigating stress, as well as stress management within this age group.

The majority of studies to date assessing the impact of palatable foods in stress coping have focused on stressors applied in adulthood. Indeed, palatable foods may also be germane to the long-term consequences of a stressors applied in childhood and adolescence. At the time of this review there were relatively few studies focusing on the juvenile time periods. In this regard, Dalmaz and colleagues investigated how access to a high fat diet modifies the short- and long-

term impact of social isolation from PD 21 to PD 28. In one study, access to a high fat diet during the pre-pubertal period did not appear to mitigate the behavioral impact of isolation on the EPM and open field behaviors during adulthood (Arcego et al., 2013). Similar results were observed when the impact of neonatal handling and social isolation in juvenility, whereby social isolation led to an anxiogenic profile in the EPM regardless of diet when rats were tested on PD 28 (Marcolin et al., 2012). Interestingly, among control rats those with access to the palatable diet displayed increased time in the open arm relative to chow-fed rats, regardless of previous exposure or non-exposure to neo-natal handling, prompting the authors to suggest that the diet in itself, may have had some anxiolytic effect (Marcolin et al., 2012). Although access to the high fat diet does not appear to mitigate the behavioral impact of social isolation, it has been proposed to prevent the short-term effects of isolation on early apoptosis and reduction of live cells in the hippocampus suggesting that the diet may be able to attenuate some physiological effects of stress (Krolow et al., 2013b). Of note, animals in the aforementioned studies are only afforded access to the palatable diet during the period of social isolation. It is possible that 7 days of access to the palatable diet was insufficient to mitigate the behavioral impact of isolation. Indeed, studies investigating the impact of maternal separation have given access to a palatable diet from weaning till sacrifice in adulthood. In one study investigating daily 3-hour maternal separation from PD 1-14, access to a palatable diet of chocolate cookies mitigated anxiety-like behaviors (e.g. decreased locomotor behavior, and fecal weight) in an activity paradigm, but had little impact on behavior in the EPM and FST, when rats were tested in adulthood (Lee, Kim, & Jahng, 2014). Access to the palatable diet was also associated with normalization of elevated basal corticosterone in rats exposed to maternal separation (Lee et al., 2014). Conversely, access to a palatable cafeteria-like diet (comprised of cakes, biscuits, dim sum and meat pies) attenuated

the long-term impact of 3-hour maternal separation between PS 2-14 in the EPM (Maniam & Morris, 2010a; Maniam & Morris, 2010b), light and dark test (Maniam & Morris, 2010a; Maniam & Morris, 2010b), as well immobility in the FST (Maniam & Morris, 2010b) and anhedonia in the sucrose preference test (Maniam & Morris, 2010a). Access to this cafeteria-style diet was associated with increased expression of hippocampal GR mRNA (Maniam & Morris, 2010a; Maniam & Morris, 2010b), attenuation of HPA-axis response to a restraint stress (Maniam & Morris, 2010b), and normalization of hypothalamic CRF mRNA (Maniam & Morris, 2010a). The documented stressor-reducing effects of palatable food in adult rats, taken together with the aforementioned results following maternal separation and the paucity of literature in the juvenile phase suggest that the potential stress-mitigating effects of high fat diets following juvenile stress warrants more attention.

Metabolic consequences of coping using food. Although self-medication with a palatable food can have some beneficial results on psychological functioning, it is likely not to be an ideal long-term stress coping strategy, from a physiological or metabolic perspective. The excess calories gained from consumption of a high fat and/or sugar diet can lead to an increase in adipose tissue (de Ferranti & Mozaffarian, 2008) which has been shown to secrete several hormones and signaling factors (Bastard et al., 2006; Kriketos et al., 2004; Woods & Seeley, 2000) involved in the regulation of food intake (Korner & Leibel, 2003; Woods & Seeley, 2000). Increases in adipose tissue and the resulting endocrinological consequences have been implicated in atherosclerosis (de Ferranti & Mozaffarian, 2008), elevated plasma levels of inflammatory cytokines (Bastard et al., 2006) and elevated concentrations of free fatty acids, which can attribute to reduced muscle glucose uptake (Randle, 1998) and the development of insulin

resistance, which is linked to type 2 diabetes, cardiovascular disease, and cancer (DeFronzo & Ferrannini, 1991; Jee, Kim, & Lee, 2005).

Obesity has become so widespread that the recent rise in the prevalence of obesity has been described as an epidemic. Although the precise cause of the 'epidemic' of obesity remains unclear, several factors are believed to play a role in the recent rise in the prevalence and incidence of obesity, including the interplay of genetics, the family environment, levels of physical activity, advertising, and sedentary behaviors (Hills, Andersen, & Byrne, 2011). However, of particular interest is the potential role of stress in the development and maintenance of obesity, especially as the level of daily stress experienced in society continues to rise (Coccurello, D'Amato, & Moles, 2009; Hill & Peters, 1998; Torres & Nowson, 2007). Approximately 50% of Canadian adults report being overweight or obese (Statistics Canada, 2012a), and nearly one quarter are classified as obese with a body mass index (BMI) greater than 30 (Statistics Canada, 2011). Obesity is associated with a number of other health problems including cardiovascular disease, type II diabetes, sleep apnea, as well as many psychosocial disorders (Knight, 2011; Stein & Colditz, 2004).

In addition to increases in obesity amongst adults, adolescent and childhood obesity has also become a concern. The relationship between stress, eating behavior and obesity is germane to the period of adolescence given the recent increases in the prevalence of obesity in this population. Approximately 20% of Canadian 12- to 17-year-olds self-report as being overweight or obese (Statistics Canada, 2012b), and 15% of children aged 5-17 years are considered obese based on age-specific BMI scales (Statistics Canada, 2012c). Recent data from the International Obesity Task Force estimated that globally approximately 200 million school aged children can be classified as being either overweight or obese (International Obesity Task Force, 2010).

Studies from around the world have shown that childhood and adolescent obesity is a strong predictor of adult obesity (Singh, Mulder, Twisk, van Mechelen, & Chinapaw, 2008; Spriijt-Metz, 2011; Whitaker, Wright, Pepe, Seidel, & Dietz, 1997). Obesity in adolescence has also been associated with adverse long-term health outcomes such as, hypercholesterolemia, insulin resistance (Shaibi & Goran, 2008), hypertension (Freedman, Dietz, Srinivasan, & Berenson, 1999), type-2 diabetes (Pinhas-Hamiel & Zeitler, 1996), non-alcoholic fatty liver disease (Cruz et al., 2005) and various cancers (Calle & Kaaks, 2004; Calle & Thun, 2004).

Indeed, the small amount of literature on access to palatable diets following juvenile social isolation and maternal separation have also highlight a trajectory to adverse metabolic consequences of high fat foods. Dalmaz and colleagues reported increased adiposity in retroperitoneal and gonadal fat pads among rats with access to a palatable diet which is further exacerbated by previous exposure to pre-pubertal social isolation (Arcego et al., 2014; Krolow et al., 2013a). Increased plasma glucose and leptin, as well as low-density lipoprotein cholesterol have also been reported (Arcego et al., 2014). Increased visceral and total fat depots, as well as increased plasma leptin and insulin have been noted in rats that experienced maternal separation and had *ad libitum* access to a palatable cafeteria diet (Maniam & Morris, 2010a; Maniam & Morris, 2010b). In addition, hyperleptinemia and reduced insulin sensitivity in adulthood have been reported among rats exposed to 24-hours of maternal deprivation and given access to a high fat diet after weaning from the dam (Mela et al., 2012).

Summary

Previous animal research on the effects of stress has focused on the prenatal, early postnatal and adult periods. The animal literature has only recently begun to shift focus towards the period of adolescence. In both humans and rodents, adolescence is a period of transition

involving changes in sociability, as well as biological and physiological changes. Stress during adolescence has consistently been linked to difficulties with depression, drug use and other psychological issues in adulthood (Compas et al., 1995; Heim & Nemeroff, 1999; Kendler et al., 1999), highlighting the importance of studying the impact of stressors during this transitional period. Indeed, current models of social and physical stress in juvenility have begun to elucidate the differences between the adult and adolescent response to stress, as well as the longer term impacts on functioning. However, there is still much work to be done, including investigating the potential mitigating effects of relevant coping behaviors. Rising levels of reported stress amid an environment where there is free access to palatable high fat and sugar foods have highlighted the impact of using foods as a means of coping with stress. Self-medication with food may be particularly relevant for youth given the recent increases in childhood and adolescent obesity, as well as the proposed sensitivity to stressors during this developmental window. The following studies were designed to further characterize the short- and long-term effects of stress exposure during juvenility and ability of palatable food to mitigate these effects. In an attempt to attain this overall objective, the following specific questions have been posed:

1) What is the impact of social stress during adulthood on anxiety and depressive symptoms? Does access to a palatable high-fat, high-sugar diet mitigate the impact of stress?

2) What is the long term impact of social stressor exposure during adolescence? Does access to a palatable high-fat, high-sugar diet mitigate the impact of a juvenile social stress?

3) What is the short and long term impact of a non-social episodic stress during adolescence? Does access to a palatable high-fat, high-sugar diet mitigate the consequences of juvenile stress exposure?

4) What is the long term impact of non-social episodic stressor exposure during adolescence when combined with social isolation via single housing conditions? Does access to a palatable high-fat, high-sugar diet mitigate the consequences of juvenile stress exposure among singly housed rats?

5) What is the long term impact of juvenile stress exposure (episodic stressor and social isolation) combined with palatable food diet on brain neurochemistry? As a relevant window to a mechanistic proxy, how might stress and diet impact the expression of dopamine receptors in the pre-frontal cortex and nucleus accumbens?

Preface to Chapter 2:

What is the impact of social stress during adulthood on anxiety and depressive symptoms? Does access to a palatable high-fat, high-sugar diet mitigate the impact of stress?

The resident-intruder model has been proposed to be an ethologically relevant model to study the impact of social stress in rodents (Miczek, 1979; Rygula et al., 2005; Wood et al., 2010). In this model, a rat (intruder) is introduced into the home cage of a larger, more aggressive rat (resident) and subjected to repeated threatening encounters. This paradigm has also been referred to as social defeat, as the intruder rat is often subject to aggressive behaviors from the resident designed to demonstrate dominance (Miczek, 1979). The typical result of these interactions involves the assumption of a submissive posture on the part of the intruder and this has been described as social defeat (Wood et al., 2010). Numerous studies have investigated the impact of this stress among adult rats. In particular, social defeat has been associated with altered HPA axis activity, increased depressive-like behaviors, promotion of anhedonia, decreased social interaction, increases in the propensity to use psychostimulants, and increased risk for cardio-vascular disease and other health related pathologies (Becker et al., 2008; Buwalda et al., 1999; Chang et al., 2009; Covington, III et al., 2005; Heinrichs et al., 1992; Miczek et al., 2004; Rygula et al., 2005; Von Frijtag et al., 2000; Wood et al., 2010; Wood et al., 2009).

In regards to the consumption of palatable foods as a means of coping with social stressors, a considerable amount of work has been done using the visible burrow system (VBS). The VSB involves a series of connecting tunnels and chambers and is thought to create ethologically relevant semi-natural social housing conditions. Indeed, within the VBS a dominance hierarchy is established and investigations of dominant and submissive rats within this paradigm have been shown to be relevant to the effects of social stress on feeding behavior. In particular, submissive

rats have been shown to display elevated glucocorticoids, low testosterone and, when allowed to recover in individual cages, are hypercorticosteronemic and hyperphagic, as well as display preferential adipose gain and glucose intolerance (Scott, Melhorn, & Sakai, 2012; Tamashiro, Sakai, Shively, Karatsoreos, & Reagan, 2011). Similar trends have been observed in humans in regards to numerous psychosocial stressors including, low socioeconomic status, personal conflicts, stressful work environments, poor self-esteem etc. (McEwen & Stellar, 1993; Steptoe et al., 2002; Wang, 2006). Amongst humans, increases in psychosocial stress have shown inconsistent effects on dietary and eating practices, with some individuals choosing to eat more, and others less. Among those who choose to increase their food intake, they often report craving higher fat and sugar foods (Scott et al., 2012). Given this, and the evidence for the negative impact of a dominance hierarchy on submissive animals in the VBS, the role of a palatable diet as a means to cope with the negative effects of social defeat is particularly interesting as it also represents a model of social stress with an inherent dominance hierarchy.

Several individuals were involved in the design and implementation of this experiment, as well as the preparation and editing of the manuscript. Drs. Zach Patterson and Alfonso Abizaid provided invaluable input regarding the design of the stressor paradigm and choice of diet, as well as provided training in the hand dissection of fat pads examined. Mr. Jonathan James assisted with data collection and the processing of endocrine and metabolic markers. Dr. Pamela Kent assisted with data analysis and manuscript edits. Dr. Zul Merali provided guidance and input during the conceptualization and design of the study, as well as on-going feedback throughout data collection, analysis and manuscript preparation. Conceptual input regarding the results of the present experiment was provided by all authors. The author of this dissertation is the first author of the present manuscript and was involved in all aspects of the design and

implementation of the experiments, as well as analysis of results and preparation of the manuscript.

Chapter 2: The impact of unpredictable chronic social defeat on ingestive behavior and behavioral markers of depression and anxiety.

Authors

J. Christine MacKay

Zachary R. Patterson

Jonathan Stewart James

Pamela Kent

Alfonso Abizaid

Zul Merali

Abstract

The Resident/Intruder paradigm is an ethologically relevant animal model of social stress which is widely used to assess the link between stress and future pathologies. The present study investigated the effects chronic unpredictable social defeat on palatable feeding, anxiety- and depressive-like behavior as measured by the EPM and FST. Caloric intake, body weight gain, glucose tolerance, and adiposity were also assessed. A 2x2 mixed factorial design was used consisting of: defeat or no defeat x palatable food or no palatable food. Male Long-Evans intruder rats were exposed to a different resident rat for a total of 60 min/day over 21 days. Control rats received daily handling. All rats had *ad libitum* access to either chow or both chow and a highly palatable food. All rats were tested 24 hours after the final defeat session in the EPM then in the FST 24 hours later. After testing, rats were given a 3 week recovery period, then FST was repeated and glucose tolerance was assessed. Access to the palatable diet led to an increased rate of weight gain, with corresponding increases in adiposity. No immediate effects of social defeat were observed in the EPM and FST. However, rats with access to the palatable diet displayed a depressive phenotype in the FST when tested 3 weeks after defeat.

Key words: Resident Intruder, Palatable Diet, Stress

Introduction

Obesity has become so widespread that the recent rise in the prevalence of obesity has been described as an epidemic. Approximately 50% of Canadian adults report themselves to be overweight or obese (Statistics Canada, 2012a), and nearly one quarter are classified as obese with a BMI greater than 30 (Statistics Canada, 2011). Obesity is associated with a number of other health problems including cardiovascular disease, type II diabetes, sleep apnea, as well as many psychosocial disorders (Knight, 2011; Stein & Colditz, 2004). Recent increases in the prevalence and incidence of obesity have been attributed to the interplay of a variety of different factors, such as genetics, the family environment, levels of physical activity, advertising, and sedentary behaviors (Hills et al., 2011). Of particular interest is the role of stress in the development and maintenance of obesity, as the level of daily stress experienced in society continues to increase (Coccurello et al., 2009; Hill & Peters, 1998; Torres & Nowson, 2007). A stressor-induced preference for palatable foods (especially those with a high fat and/or sugar content) has been documented in both animals and humans (Dallman, 2010; Gibson, 2006; O'Connor et al., 2008; Zellner et al., 2006).This preference is proposed to serve as a form of self-medication in order to protect against the effects of stress (Dallman et al., 2005).

Given the interplay between stress and obesity, animal models with external validity are of particular importance. The resident-intruder model has been proposed to be an ethologically relevant model to study the impact of social stress in rodents (Miczek, 1979; Rygula et al., 2005; Wood et al., 2010) as well as the impact of status in a social hierarchy (Solomon et al., 2007). In this model, a rat (intruder) is introduced into the home cage of a larger, more aggressive rat (resident) and subjected to repeated threatening encounters. This paradigm has also been referred to as social defeat, as the intruder rat is often subject to aggressive behaviors from the resident

designed to demonstrate dominance (Miczek, 1979). The typical result of these interactions involves the assumption of a submissive posture on the part of the intruder (e.g. social defeat) (Wood et al., 2010). Numerous studies have investigated the impact of this stress among adult rats. In particular, social defeat has been associated with altered HPA axis activity, increased depressive-like behaviors, promotion of anhedonia, decreased social interaction, increases in the propensity to use psychostimulants, and increased risk for cardio-vascular disease and other health related pathologies (Becker et al., 2008; Buwalda et al., 1999; Chang et al., 2009; Covington, III et al., 2005; Heinrichs et al., 1992; Miczek et al., 2004; Rygula et al., 2005; Von Frijtag et al., 2000; Wood et al., 2010; Wood et al., 2009).

Investigations of the impact social defeat on metabolic processes have demonstrated an increase in total caloric intake among defeated animals relative to non-stressed controls (Patterson et al., 2013). Among defeated rats, submissive animals within the hierarchy show a greater increase in high fat diet intake relative to dominant animals (Moles et al., 2006), as well as increases in body mass that are independent of food intake and last weeks after the last defeat session (Patterson et al., 2013; Solomon et al., 2007). Elevations in blood glucose, corticosterone and visceral adiposity have also been noted (Lutter et al., 2008; Patterson & Abizaid, 2013; Patterson et al., 2013; Solomon et al., 2007). It is noteworthy that the physiological changes following defeat vary depending on whether measurement is done immediately after defeat (Bartolomucci et al., 2004; Bhatnagar & Vining, 2003; Foster et al., 2006; Keeney et al., 2006; Lutter et al., 2008) or after a recovery period where the animals has been removed from the stressful environment (Chuang et al., 2011; Keeney et al., 2006; Lutter et al., 2008; Patterson, Parno, Isaacs, & Abizaid, 2013; Solomon et al., 2007). Similar trends have been observed in humans in regards to numerous psychosocial stressors including, low socioeconomic status,

personal conflicts, stressful work environments, poor self-esteem etc. (McEwen & Stellar, 1993; Steptoe et al., 2002; Wang, 2006). Amongst humans, increased psychosocial stress yields inconsistent effects on dietary and eating practices, with some individuals choosing to eat more, and others less. Among those who choose to increase their food intake, higher craving for fat and sugary foods has been reported (Scott et al., 2012).

The present study investigated that impact of chronic unpredictable social defeat on depressive- and anxiety-like behaviors. A secondary objective was to evaluate the potential stress-reducing effects of a high-fat diet and accompanying metabolic consequences given previous evidence that submissive animals will increase their intake of a high fat diet.

Materials and Methods

Animals. Twenty-eight 50 day old male Long Evans rats (200-225g) were used as intruders. Sixteen Long Evans retired breeders (600g) were used as residents. All rats were obtained from Charles River (Quebec, Canada) and single housed in standard plastic cages (37cm x 26 cm x 18cm) with bedding at a room temperature of 22±1°C on a 12 hour light-dark cycle (lights on at 0700hrs). Weight gain was measured on postnatal day (PD) 50, 60, 70, 80, 90 and 100. All procedures met the guidelines established by the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Ottawa Institute of Mental Health Research.

Experimental design. An overview of the stress procedure and testing schedule is presented in Figure 1. Upon arrival rats were randomly assigned to one of the following groups: Chow + No Stress; Chow + Stress; Palatable + No Stress; and Palatable + Stress. All animals underwent all behavioral tests and glucose tolerance testing.

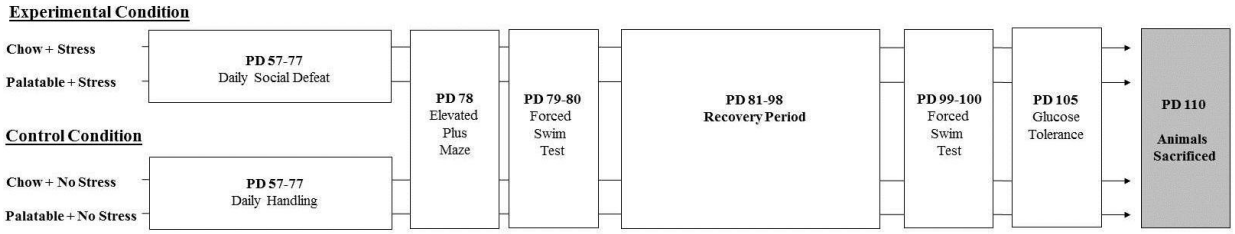

Figure 1. Summary of Study Design. Animals assigned to stress condition were exposed to a different resident rat per day from PD 57-77 while control animals received daily handling. All animals were tested in the indicated behavioral paradigms as described.

Diet. All rats were given *ad libitum* access to standard laboratory chow (3.4 kcal/g, 4.5% fat, 18.1% protein, 57.3% carbohydrate, Charles River Rodent Diet 5075, Agribrand Purina Canada, Woodstock, Ontario, Canada) and rats assigned to the palatable food condition were given *ad libitum* access to the 45%Kcal Fat diet (4.7 Kcal/g, 23.2% fat, 17.3% protein, 47.6% carbohydrates, TD.08811, Harlan Laboratories, Madison, Wisconsin, USA). Every 7 days, food consumption was measured by subtracting the weight of the food from the food weight 24-hours earlier. Total caloric intake was calculated by multiplying the energy content (Kcal/g) of individual diets by the amount consumed. Preference for palatable food was calculated by taking the ratio of calories of palatable food consumed over total calories consumed. Food intake data was collected from PD 57 to PD 99.

Resident intruder paradigm. Rats in the social defeat condition (intruders) were introduced into the home cage of a different resident daily for 21 days (PDs 57-77). Resident/Intruder interactions took place at a different time each day. Intruders and residents were allowed to interact for a maximum of 10 min then a plastic holed-divider was used to separate the rats. Interactions were terminated before the 10 min mark if the experimenter

suspected that fights could result in injury. Following separation, the intruder remained in the resident's cage for the remainder of the time allotted for the test session (60 min including the 10 min interaction). This prevented further physical contact between the resident and intruder while maintaining olfactory and visual cues and thus maintaining the psychosocial nature of the stress (Rygula et al., 2005).

Behavioral testing. Behavioral testing was conducted under low illumination (30 - 40 lux) between 10:00-13:00 daily following a 1 hr. period during which rats habituated to the testing room. Behavior was monitored via a video camera mounted above the arena.

Elevated plus maze. On PD 78, rats were placed on the center platform of the elevated plus maze (EPM), facing a closed arm. The EPM consisted of two open arms (50 x 10 cm) and two perpendicularly situated arms enclosed by 40 cm high walls, elevated approximately 66 cm above the floor. Behaviors scored during the 5 min test included time spent on the open and closed arms, risk assessment behavior (unprotected head dips; head protruding over the edge of an open arm) and number of entries into the closed arms. Time in the open arms and unprotected head dips are validated measures of reduced anxiety-like behavior (Carobrez & Bertoglio, 2005). The number of entries into the closed arms of the maze are viewed as a measure of spontaneous locomotor activity (Walf & Frye, 2007).

Forced swim test. The forced swim test is a widely used behavioral despair paradigm used to evaluate the effectiveness of antidepressant drugs (Porsolt et al., 1978; Porsolt, Bertin, & Jalfre, 1977; Porsolt et al., 1977; Schiller, Pucilowski, Wienicke, & Overstreet, 1992). The forced swim arena consisted of a clear Plexiglas cylinder (20 x 45 cm, water depth 30 cm, temperature 25 °C; Stoelting Co., Wood Dale, Illinois). Rats were initially test in the FST on PD 79-80 and then a second test session was completed on PD 99-100. On PD 79 and 99, a

habituation session (15 min) was performed twenty-four hours prior to the test session. On test day (PD 80 and 100) the animal was returned to the same tube for 5 minutes. The time spent immobile was recorded during the test session. Immobility was defined as an animal floating in the water without struggling, and only engaging in movements to keep the head above water (Slattery & Cryan, 2012). Increases in immobility are thought to reflect behavioral despair (Cryan, Markou, & Lucki, 2002; Lucki, 1997). Therefore reduced immobility is used to assess the antidepressant properties of a given compound (Cryan et al., 2005).

Corticosterone response to a swim stress. On PD 99, the 15 min habituation session of the forced swim test was used to assess the animals' corticosterone response to a stressor challenge. Blood samples, collected using tail venipuncture, were taken immediately before the stressor, and 5, 15, 30, and 60 min post-stressor exposure. Blood droplets were deposited onto 903 Protein Saver filter paper (GE Healthcare Bio-Sciences Corp, MA, USA), allowed to dry at room temperature then stored at -20°C.

Collected blood droplets were analyzed for whole blood corticosterone levels using commercial radioimmunoassay (RIA) kits (MP biomedical, CA, USA). Two days prior to the RIA procedure, blood was eluted from the filter paper by placing one 3 mm punch (per time point) of filter paper in a 12 x 75 culture tube containing 200 µL Dulbecco's Phosphate Buffered Saline (Sigma, item D-5773) w/0.1% gelatine, covered with parafilm in a fridge at 4°C. On the day of the RIA procedure, culture tubes containing the samples were placed on an orbital shaker for 1 hour at room temp. Corticosterone levels were the determined from the eluted blood sample using the commercial RIA kits as per the manufacturer's instructions. The inter- and intra-assay variability was 6.83% and 1.62%, respectively.

Glucose tolerance test. On PD 105, following 12 hr of fasting, all rats were orally administered with a 0.75g/mL dextrose solution (dose: 1.75g/kg; via gavage). Blood glucose was measured by applying a drop of blood (via tail venipuncture) onto a test strip, then taking a reading with a blood glucose meter (Accu-Chek Aviva Nano, Roche Diagnostics, Mannheim, Germany). Levels were assessed immediately before the administration, and 10, 30, 60 and 120 min post-injection.

Adiposity. On PD 110, animals were sacrificed and carcasses immediately stored at -20°C. At a later date the carcasses were thawed at 4°C and fat pads hand dissected and weighed. The fat pads collected included: mesenteric, retroperitoneal, subcutaneous inguinal white fat, and inter-scapular brown fat.

Statistical analysis. Data obtained from the EPM, FST, and adiposity were analyzed by 2-way analysis of variance (ANOVAs) where Diet (2 levels; chow vs. palatable food) and Stress (2 levels; stress vs no stress) were the between group factors. Body weight was analyzed using 3-way repeated-measures ANOVAs, where Diet (2 levels) and Stress (2 levels) were the between group variables and Time (9 points) the repeated within group variable. Total caloric intake, corticosterone response to a swim stress and glucose tolerance results were analyzed using 3-way repeated-measures ANOVAs, where Diet (2 levels) and Stress (2 levels) were the between group variables and Time (5 points) the repeated within group variable. Calories from the palatable diet was analyzed using 2-way repeated-measures ANOVAs, where Stress (2 levels) was the between group variable and Time (5 points) the repeated within group variable.

Subsequent follow-up comparisons were conducted using *t* tests with a Bonferroni correction to maintain the alpha level at .05. For some measures a priori predictions that access to the palatable diet would alter the impact of the stressor were made. Analyses of the simple

effects for interactions and a priori predictions were conducted irrespective of whether the F value for an interaction reached significance (Winer, 1962), especially when visual inspection of graphed results suggested that a significant difference may exist. Data points ±3 standard deviations from calculated means were considered as outliers and not included in statistical analysis (Taylor, 1997). Some rats were removed from the statistical analysis of variable due to missing data, thus the N and df associated with these measures vary across outcomes.

Results

Impact of social defeat and diet on body weight and feeding behavior. Three-way repeated measures ANOVA revealed significant main effects for Diet x Time, $F_{Diet\ x\ Time}(5,120) = 19.05$, $p < .001$ and Stress x Time, $F_{Stress\ x\ Time}(5,120) = 4.48$, $p = .001$ (Figure 2A) on body weight. The interaction between Diet x Stress x Time was non-significant, $F_{Stress\ x\ Diet\ x\ Time}(5,120) = .212$, $p = .957$. Simple effects analyses were completed based on an a priori hypothesis that a significant interaction would be present. Simple effects analyses demonstrated that all animals with access to the palatable diet weighed more than Chow fed rats from PD 70 onwards. A significant effect of stress was observed on PD 70 and 80 where defeated rats weighed less than non-stressed controls; however, this was only observed in rats with access to the palatable diet.

In terms of total caloric intake, a significant effect of Stress x Time was observed, $F_{Stress\ x\ Time}(7,168) = 11.66$, $p < .001$; Figure 2B. In general, defeated rats consumed fewer calories relative to their controls. The interaction between Stress x Diet x Time was non-significant, $F_{Stress\ x\ Diet\ x\ Time}(7,168) = .488$, $p = .843$, as well as Diet x Time, $F_{Diet\ x\ Time}(7,168) = 1.09$, $p = .369$. Follow-up simple effects analyses were completed on the assumption that a significant interaction would be present. Among non-stressed controls access to the palatable diet was

associated with increased caloric intake from baseline until the final week of the resident intruder paradigm. All defeated rats consumed fewer calories during the first week of resident/intruder exposure regardless of diet. This effect persisted into the second week of defeat for those rats with access to the palatable diet. No significant effect of Stress x Time was observed in preference for the palatable food, $F_{Stress\ x\ Time}$ (7,84) = 1.41, p = .21; Figure 2C.

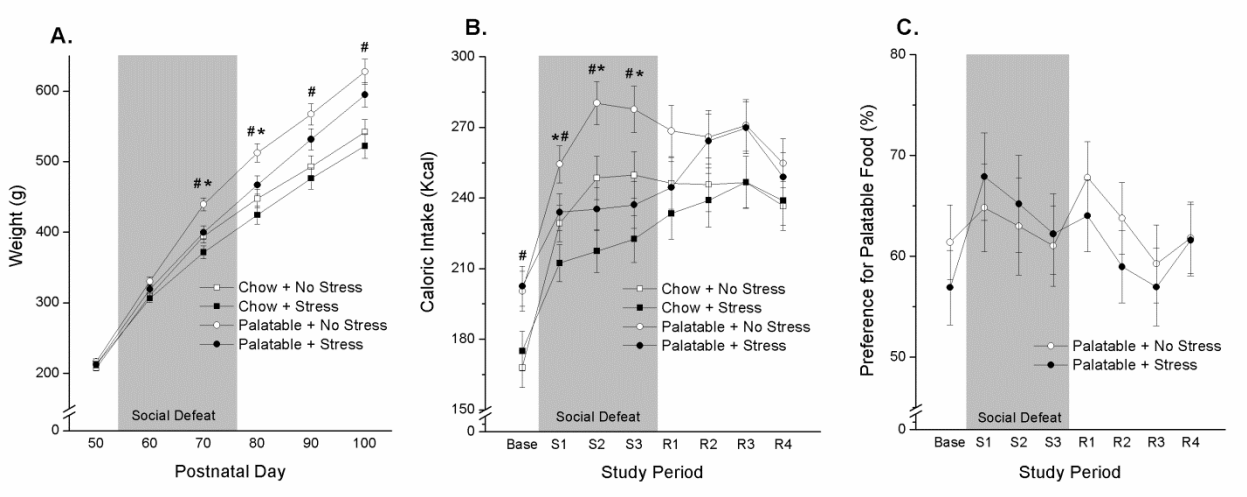

Figure 2. Effects of social defeat and diet on weight, caloric intake and preference for a palatable diet. Overall, rats with access to the palatable diet weighed more than chow fed rats (A). However, during the last two weeks of resident/intruder interactions (PD 70 and 80) defeated rats with access to the palatable diet gained weight at a slower pace relative to their controls. Defeated rats were observed to consume fewer calories during the last two weeks of resident/intruder exposures (B). The experience of social defeat did not appear to impact preference for the palatable food (C). Data represents mean ± standard error of the mean (SEM). * Significant effect of Stress at $p < .05$; # Significant effect of Diet at $p < .05$

Table 1

Impact of social defeat and diet on behavior in the EPM

	Chow+No Stress	Chow+Stress	Palatable+No Stress	Palatable+No Stress
Time spent in open arms	106.21 ± 16.81	110.07 ± 16.81	95.99 ± 16.81	123.80 ± 16.81
Time spent in closed arms	120.67 ± 14.06	88.69 ± 14.06*	119.84 ± 14.06	94.41 ± 14.06*
Entries into closed arm	7.00 ± .73	6.00 ± .73	7.00 ± .73	6.14 ± .73
Number of risk assessments	2.27 ± .89	2.43 ± .89	2.43 ± .89	5.86 ± .89*

Mean ± SEM
* Significant effect of Stress at $p < .05$.

Impact of social defeat and diet on behavioral markers of depression and anxiety. A significant Stress effect was observed on time spent in the closed arm of the EPM as exposure to defeat was associated with decreased time in the closed arms, $F_{Stress}(1,27) = 4.18$, $p = .05$; however, the main effect of Diet, $F_{Diet}(1,27) = .029$, $p = .866$ and the Diet x Stress interaction, $F_{Stress\ x\ Diet}(1,27) = .053$, $p = .820$, were non-significant. No main effects for Stress, $F_{Stress}(1,27) = .887$, $p = .356$, and Diet, $F_{Diet}(1,27) = .011$, $p = .918$, were observed in time spent in the open arm of the EPM and the interaction between Stress and Diet was non-significant, $F_{Stress\ x\ Diet}(1,27) = .508$, $p = .48$. Two-way ANOVA for number of risk assessments made approached significance for both the effects of Stress, $F_{Stress}(1,27) = 4.01$, $p = .057$ and Diet, $F_{Diet}(1,27) = 4.01$, $p = .057$; however, the interaction between Stress and Diet was non-significant, $F_{Stress\ x\ Diet}(1,27) = 3.39$, $p = .078$. No significant effects of Stress, $F_{Stress}(1,27) = .340$, $p = .565$ and Diet, $F_{Diet}(1,27) = .340$, $p = .565$, were observed in terms of the number of entries into the closed arm of the EPM. Similarly, no interaction between Stress and Diet was observed in the number of closed arm entries $F_{Stress\ x\ Diet}(1,27) = .604$, $p = .45$; suggesting no differences in locomotor

behavior between groups. Follow-up post hoc comparisons were completed on EPM measures based on an a priori hypothesis that significant interactions would be present. Simple effects analyses indicated that exposure to defeat was associated an increase risk assessment, but only in rats with access to Palatable Food (Table 1). All other post-hoc comparisons were non-significant.

The impact of social defeat and diet on time spent immobile in the FST is depicted in Figure 3. Two-way ANOVA revealed no significant main effects of Stress, $F_{Stress}(1,22) = .232$, $p = .653$, and Diet, $F_{Diet}(1,22) = .3.21$, $p = .087$, on time spent immobile, in the FST on PD 80. The interaction between Stress and Diet was also non-significant, $F_{Stress\ x\ Diet}(1,22) = .065$, $p = .80$, Figure 3A. When rats were re-tested in the FST on PD 100 (Figure 3B), a significant effect of Diet was observed in the time spent immobile, $F_{Diet}(1,22) = 9.16$, $p = .006$. On PD 100, all rats with access to the palatable food spent more time immobile compared to Chow fed rats. The main effect of Stress, $F_{Stress}(1,22) = .016$, $p = .902$, and interaction between Stress and Diet, $F_{Stress\ x\ Diet}(1,22) = .125$, $p = .727$, was non-significant.

Following a significant diet effect in the FST, a Pearson's *r* was computed to assess the relationship between body weight on PD 100 and immobility in the FST. The relationship between the sum of fat pads and immobility in the FST was also assessed. A significant moderate positive correlation was observed between fat pad weight and time spent immobile, $r26 = .397$, $p = .044$. In general, increased fat pad weight was associated with an increase in time spent immobile. Body weight on PD 100 was not correlated with immobility in the FST, $r(26) = .336$, $p = .093$.

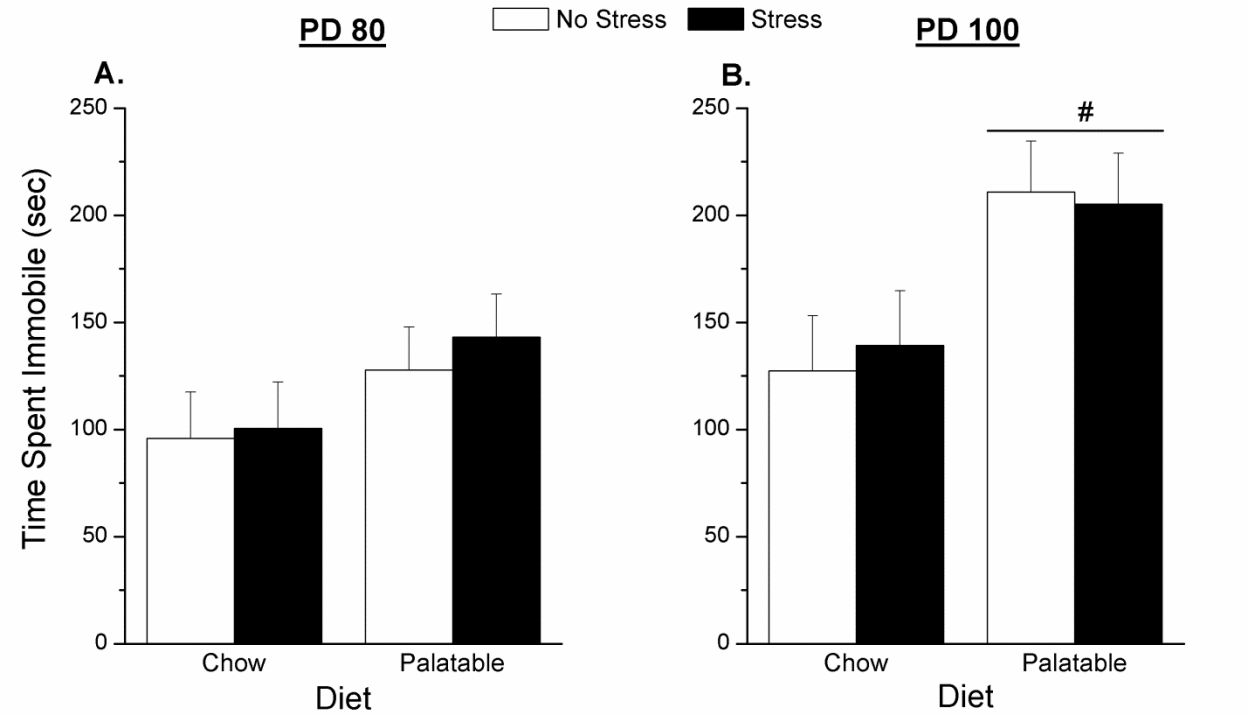

Figure 3. Impact of social defeat and diet on immobility in the FST. No significant effects of Diet and Stress were observed in the FST one week after resident/intruder interactions. When re-tested in the FST two weeks later, rats with access to the palatable diet appeared to display increased learned helplessness in the FST. Bars represent mean ± SEM. # Significant effect of Diet at $p < .05$

Impact of social defeat and diet on endocrine and metabolic indices. Three-way repeated measures ANOVA revealed no significant interaction between Stress x Diet x Time on corticosterone response to the swim stressor, $F_{Stress\ x\ Diet\ x\ Time}$ (5,120) = 2.01, p = .10; Figure 4A. No significant effect of Diet x Time, $F_{Diet\ x\ Time}$ (5,120) = .469, p = .758, and Stress x Time, $F_{Stress\ x\ Time}$ (5,120) = .690, p = .610, was observed. Post-hoc simple effects analyses were completed based on an a priori hypothesis that a significant Stress x Diet x Time interaction would be present. Simple effects analysis revealed a significant effect of stress on all time points;

however this was only observed in rats with access to the palatable diet. No significant effects of Diet x Time, $F_{Diet\ x\ Time}$ (4,96) = .174, p = .951,and Stress x Time, $F_{Stress\ x\ Time}$ (4,96) = .535, p = .711, were observed in terms of the rats' ability to tolerate a glucose load. The interaction between Diet x Stress x Time was also non-significant, $F_{Stress\ x\ Diet\ x\ Time}$ (4,96) = 1.91, p = .116; Figure 4B.

Access to the palatable diet significantly increased the weight of all fat pads dissected (Figure 4C and D). A significant Diet effect was observed in the sum of all fat pads, F_{Diet} (1,27) = 41.78, p < .001. Stress alone did no significantly influence total fat pad weight, F_{Stress}(1,27) = 1.74, p = .200, and the interaction between Stress and Diet was non-significant, $F_{Stress\ x\ Diet}$(1,27) = .722, p = .404. A significant effect of Diet was observed across all the individual fat pads including; mesenteric, F_{Diet}(1,27) =29.59, p < .001; retroperitoneal, F_{Diet} (1,27) = 11.53, p = .002; subcutaneous fat, F_{Diet}(1,27) = 37.16, p < .001 and brown fat, F_{Diet} (1,27) = 13.85, p = .001. The main effect of Stress and interaction between Stress and Diet were non-significant for all individual fat pads: mesenteric, F_{Stress}(1,27) =2.59, p = .120 and $F_{Stress\ x\ Diet}$ (1,27) =.868, p = .361; retroperitoneal, F_{Stress}(1,27) =.415, p = .525 and $F_{Stress\ x\ Diet}$ (1,27) =.832, p = .371; subcutaneous fat, F_{Stress}(1,27) =.712, p = ..407 and $F_{Stress\ x\ Diet}$ (1,27) =.348, p = ..561; and brown fat, F_{Stress}(1,27) =.066, p = .799 and $F_{Stress\ x\ Diet}$ (1,27) =.276, p = .604.

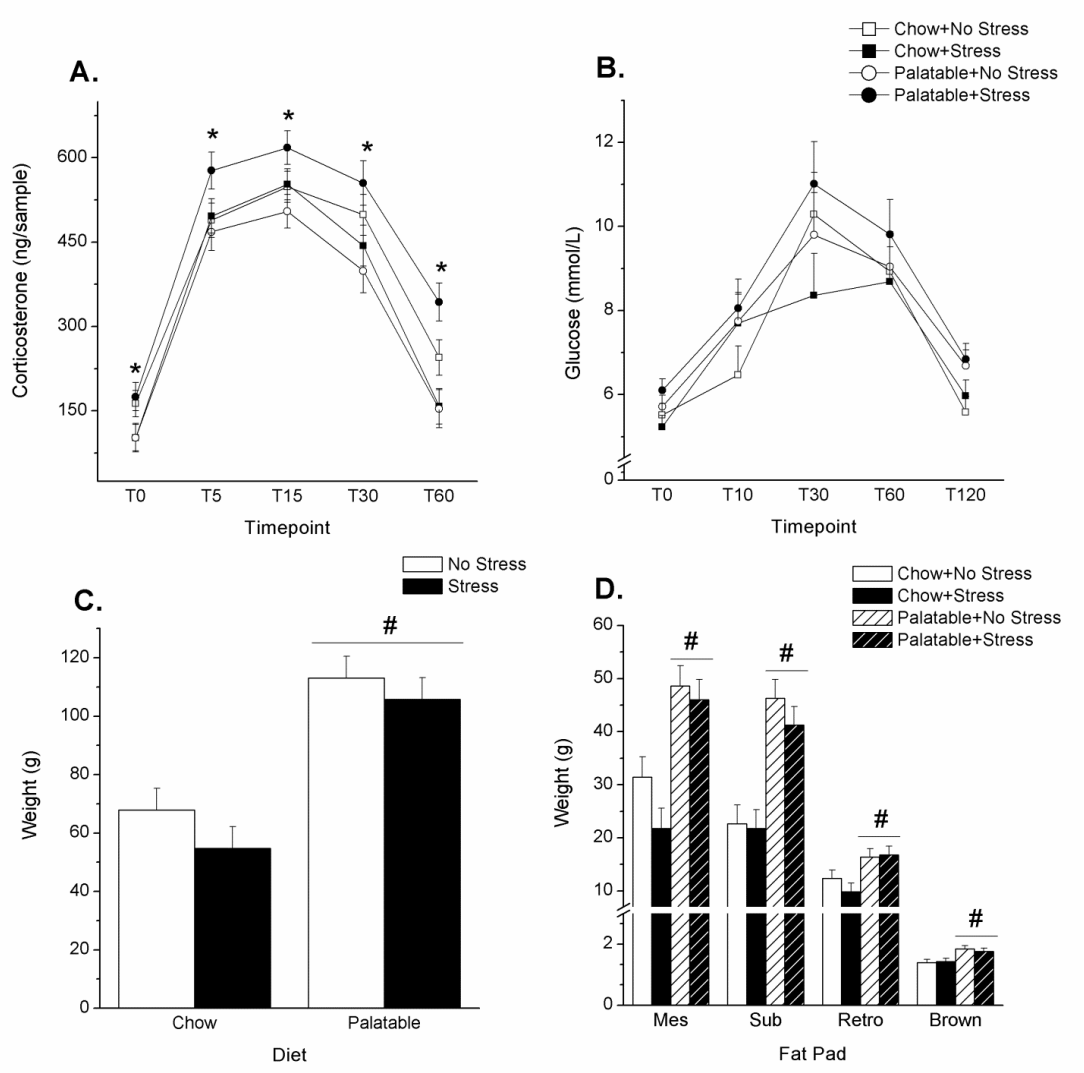

*Figure 4.*Impact of social defeat and diet on corticosterone and metabolic indices. In response to a swim stress, defeated rats with access to the palatable diet displayed increased basal corticosterone relative to non-stressed controls (A). Access to the palatable diet resulted in a general increase in the weight of all fat pads irrespective of previous stressor exposure (C and D). No significant differences in the ability to tolerate a glucose load were observed (B). Mes = Mesenteric fat pad. Retro = retroperitoneal fat pad. Brown = Inter-scapular brown fat. Sub = Subcutaneous inguinal white fat pad. Data represents mean ± SEM. * Significant effect of Stress at $p < .05$; # Significant effect of Diet at $p < .05$;

Discussion

The present study investigated the immediate and delayed effects of social defeat on behavioral indices of depression and anxiety. As expected, total caloric intake was reduced during the resident intruder paradigm in both groups exposed to the stressor. While no significant differences in body weight were observed among defeated rats with access to the Chow only, reduced weight gain following social defeat has been well established (Berton et al., 1998; Bhatnagar & Vining, 2003; Bhatnagar et al., 2006; Haller et al., 1999; Meerlo et al., 1996; Ruis et al., 1999). It was anticipated that defeat rats with access to Chow only would display a reduction in weight gain. Indeed, their food intake was reduced during the defeat period; however the relationship between food intake and rate of weight gain may not be simple. Reduced caloric efficiency following chronic defeat has been previously reported (Bhatnagar et al., 2006) suggesting that exposure to a social stressor may result in changes in metabolic functioning. Reduced caloric efficiency has also been observed in other stress paradigms (Pecoraro et al., 2004). Nonetheless, differential responses between food intake and weight gained are observed across studies and responses appear to vary based on the stressor employed (Akana et al., 1996; Harris et al., 2002). Interestingly, a corresponding reduction in the rate of weight gain was also observed in defeated rats with access to the palatable diet relative to their controls. However, the rate of weight gain among defeated rats increased during the recovery period such that there was no significant difference in body weight at the time of sacrifice. Although no preference for the palatable diet was observed, total caloric intake among defeated rats was comparable to controls during the recovery period. It is possible that the normalization of caloric intake during recovery may have had a corresponding effect on rate of weight gain.

There is considerable variability in the literature in terms of how the resident intruder paradigm is employed, from single defeat sessions (Haller et al., 1999) to longer-term exposure (Berton et al., 1998; Bhatnagar & Vining, 2003; Bhatnagar et al., 2006; Rygula et al., 2005). Nonetheless, social defeat has been shown to have impact on behavioral measures. Reduced exploratory and depressive-like behavior has been previously reported following five weeks of daily social defeat (Rygula et al., 2005). Similar results, in conjunction with increased anxiety-like behavior and memory impairment, have been reported following seven days of defeat (Patki et al., 2013). Behavioral changes following social defeat have also been noted to have long-lasting consequences (Hollis et al., 2010; Koolhaas, Meerlo, De Boer, Strubbe, & Bohus, 1997; Meerlo et al., 1996; Ruis et al., 1999). In our study, social defeat appeared to have no immediate impact on anxiety- and depressive-like behavior measured by the EPM and FST. This may be the result of limitations regarding our resident intruder protocol. In the present study, residents were not pre-screened for consistent aggressive behavior. This was due to limited availability of resident rats (n=18), and reports that approximately half of screened residents do not meet the criteria for study inclusion (B. Bingham, personal communication, May 31, 2011; Patki et al., 2013). While resident screening is not likely to remove all variability, set parameters (e.g. attack intruder within 3 min) allows for more consistency. Throughout the course of the experiment levels of aggression among residents fluctuated. While this may be the result of habituation to the intruder encounters (Patki et al., 2013), some resident were observed to attempt to isolate themselves from the intruder rat. The chronic nature of the present study (21 days) and exposure to multiple intruders may have been equally stressful for resident rats and contributed to the apparent "burn out" observed. Taken together, the aforementioned limitations likely resulted in a large degree of variability in terms of stressor exposure among intruder rats. While intruders

were exposed to a novel resident each day, the variability in levels of aggression may have confounded the potency of the stressor resulting in an inconsistent "dose" of defeat across intruders.

While no immediate effects of social defeat were observed, a significant diet effect emerged in the FST at the later time point. In general, rats with access to the palatable diet displayed less activity in the FST suggesting an apparent depressive profile. There is some evidence to suggest that chronic dietary fat may serve as a potential stressor. Exaggerated HPA-axis function with corresponding depressive-like behavior in FST has been previously reported among non-stressed animals with free-access to a high-fat diet (Park, Kim, Lee, & Jhang, 2014; Soulis, Papalexi, Kittas, & Kitraki, 2007; Tannenbaum et al., 1997). However, the potential compounding effects of water depth, body weight and fat suggest an alternate interpretation of the FST results. This is in part due to the observation that rats with access to the palatable diet were much larger than Chow-fed rats. This introduced confounds into the FST procedure. While we followed the suggested protocol in the literature (Porsolt et al., 1978; Porsolt, Bertin, & Jalfre, 1977; Porsolt et al., 1977; Schiller, Pucilowski, Wienicke, & Overstreet, 1992), the water depth (30 cm) did not appear to be sufficient to allow for true learned helplessness. Indeed, the larger rats appeared able to prop themselves up on their tails, which may have facilitated immobility. This phenomenon has been previously reported when water depth is insufficient (Slattery & Cryan, 2012). The significant correlation between fat pad weight and time spent immobile raises a question regarding the impact of adiposity on behavior in the FST.

The potential mitigating effects of the palatable diet on impact of social defeat were difficult discern given the observed results of the present study. While a stress-induced preference for palatable foods has been previously reported (Dallman, 2010; Gibson, 2006;

O'Connor et al., 2008; Zellner et al., 2006), no apparent preference was observed. Furthermore, we did not see the hypothesized reduction in endocrine activity among rats with access to the palatable diet. Defeated rats with access to the palatable diet displayed an increased basal corticosterone levels which carried through to all time points in the corticosterone challenge. These results are inconsistent with the current literature documenting the apparent dampening effects of palatable foods on HPA axis activity (Bhatnagar et al., 2000; Christiansen et al., 2011; Dallman et al., 2003a; Dallman et al., 2003b; Laugero, Bell, Bhatnagar, Soriano, & Dallman, 2001; Levin, 1996; Michel, Levin, & Dunn-Meynell, 2003; Ortolani, Garcia, Melo-Thomas, & Spadari-Bratfisch, 2014; Pecoraro et al., 2004; Strack et al., 1997; Zeeni et al., 2012). There is some evidence to suggest that free access to high-fat diets alone can negatively impact both basal and stress-induced levels of adrenocorticotropic hormone and corticosterone (Tannenbaum et al., 1997; Willner, Muscat, & Papp, 1992a). However, controls rats with access to the palatable diet did not display altered HPA-axis functioning. Nonetheless, all rats displayed increases in adiposity in all fat pads measured. Increased adiposity is a well-established consequence of high-fat and high-sugar diets (Seidell, 1998).

In summary, the present study draws attention to the potential impact of methodological issues in the resident intruder paradigm. Namely, the importance of resident screening to ensure maximum consistency of stressor exposure across intruders, as well as consideration for the impact of the paradigm on levels of stress in the residents. Furthermore, our FST results highlight the potential confounding effect of adiposity on activity level within this paradigm. This warrants further exploration given the well documented impact of palatable foods on body weight and adiposity and the body of evidence suggesting that a palatable high fat/high sugar diet induces a depressive phenotype based on results obtained using the FST.

Disclosure

All of the authors declare that they have no direct or indirect conflicts of interests of a financial nature relevant to the present work.

Acknowledgements

This project was funded by an operating grant from the Canadian Institute for Health Research (CIHR) awarded to Zul Merali, and by a CIHR doctoral Canada Graduate Scholarship awarded to J. Christine MacKay.

Preface to Chapter 3:

What is the long term impact of social stressor exposure during adolescence? Does access to a palatable high-fat, high-sugar diet mitigate the impact of a juvenile social stress?

Stressor exposure may be particular germane to the period of adolescence as this period is characterized by social challenges including the search for autonomy, an increase in child-parent conflict, as well as a shift in social interaction from familial to peer relationships (Panksepp, 1981; Spear, 2000). One area of interest is the implications of bullying as a source of stress. Among Canadians, 38% of males and 30% of females reported having experienced occasional or frequent bullying during their childhood and adolescence (Kim & Leventhal, 2008). The use of the resident intruder paradigm in juvenility has been proposed to be model of social stress that may approximate the phenomenon of bullying. While there have been a number of studies investigating the impact of the resident intruder paradigm in adult rats, the literature in the realm of juvenility is continuing to develop. In this regard, juvenile social defeat has been associated with an decreased social interaction and exploration behavior in adulthood (Vidal et al., 2007; Watt et al., 2009), increases in proactive coping behavior (Bingham et al., 2011), and increased anxiety-like behavior in adulthood (McCormick & Mathews, 2010; McCormick et al., 2005; McCormick et al., 2008; Vidal et al., 2007; Watt et al., 2009). However, more recent evidence suggests that juvenile social defeat may not necessarily result in a lasting increased vulnerability to later stressful challenges suggesting that adolescents may be more resilient to the long-term impacts of stress than previously thought (Buwalda et al., 2013).

Despite some conflicting findings of the impact of juvenile social defeat, the potential stress mitigating role of a palatable diet amidst social stress during adolescence has yet to be fully characterised. There is some evidence to suggest that access to a palatable diet may have

mitigating effects on the impact of early life stress. In this regard, Maniam and Morris (Maniam & Morris, 2010a) demonstrated that the long-term negative effects of an early life stressor (maternal separation) were normalized by the provision of a palatable high fat diet. There is also evidence to suggest that access to a palatable diet can mitigate the impact of stressor exposure in adulthood stressor (Buwalda et al., 2001; Christiansen et al., 2011; Fachin et al., 2008; Kant & Bauman, 1993; la Fleur et al., 2005; Pecoraro et al., 2004; Ulrich-Lai et al., 2007; Ulrich-Lai et al., 2010; Ulrich-Lai et al., 2011; Young, 2000). However, the stress-reducing effects of a palatable diet often come at the cost of negative changes in adiposity and metabolic functioning (Bastard et al., 2006; de Ferranti & Mozaffarian, 2008; Korner & Leibel, 2003). It is noteworthy, that responses to stressors differ between adolescent and adult rats (Avital & Richter-Levin, 2005; Bingham et al., 2011; Romeo, 2010). Furthermore, there has been a documented increase in the rates of childhood obesity (International Obesity Task Force, 2010). Taken together, the increased stress observed in adolescence and the rise of childhood obesity suggests that the investigation of the stress-buffering effects of palatable foods may be particularly germane to this developmental period.

Several individuals were involved in the design and implementation of this experiment, as well as the preparation and editing of the manuscript. Mr. Jonathan James assisted with data collection and the processing of endocrine and metabolic markers. Mr. Christian Cayer assisted with the analysis of data collected from behavioral assays. Dr. Pamela Kent assisted with data analysis and manuscript edits. Dr. Zul Merali provided guidance and input during the conceptualization and design of the study, as well as on-going feedback throughout data collection, analysis and manuscript preparation. Conceptual input regarding the results of the present experiment was provided by all authors. The author of this dissertation is the first author

of the present manuscript and was involved in all aspects of the design and implementation of the experiments, as well as analysis of results and preparation of the manuscript.

Chapter 3: Ability of palatable food consumption to buffer against the short and long term behavioral consequences of social defeat exposure during juvenility in rats

Authors

J. Christine MacKay

Jonathan S. James

Christian Cayer

Pamela Kent

Zul Merali

Abstract

In adult rats, access to a palatable diet has been suggested as a form of self-medication that can mitigate the effects of stressors. Approximately 10% of all adolescents are repeatedly victimized by their peers raising a possibility that palatable food consumption may be relevant to this developmental window. The purpose of this study was to assess the long-term impact of juvenile social defeat and whether the associated negative consequences could be attenuated by palatable food consumption. A 2x2 mixed factorial design was used consisting of: stress or no stress x palatable food or no palatable food. Juvenile Sprague-Dawley rats received daily exposure to a different adult resident rat from postnatal day (PD) 28-34. Control rats received daily handling. All rats had *ad libitum* access to either chow or both chow and the palatable food commencing upon arrival (PD 21). Anxiety- and depressive-like behavior was assessed in early adulthood (PD 59) using the open field, elevated plus maze, social interaction test and forced swim test. Results suggest that exposure to social defeat during juvenility resulted in a mild anxiogenic profile in the open field amongst rats with access to chow only. Defeated rats with access to chow only also displayed increased social interaction as adults. Interestingly, access to the palatable food altered behavioral responses during juvenile defeat sessions. Specifically, palatable food-fed rats displayed significantly longer latencies to submit to the resident and less time in a submissive posture.

Keywords: Juvenile social defeat, Anxiety, Obesity, High fat diet, Palatable Diet, Resident Intruder

Introduction

Exposure to stress had been identified as important precipitating factor in the development and maintenance of numerous psychiatric disorders including anxiety and mood disorders, as well as psychotic disorders (Heim & Nemeroff, 1999; Kendler et al., 1999; van et al., 2008). Individuals may be particularly sensitive to stress during certain developmental windows. For example, prenatal or neonatal stressor exposure can have profound and enduring effects on behavior and neurochemical functioning (Graham, Heim, Goodman, Miller, & Nemeroff, 1999; Weinstock, 1997). In a similar vein, adolescence represents a unique period of stressor vulnerability given that regions related to emotional and learning process, such as the prefrontal cortex, hippocampus, and the amygdala, all undergo substantial remodeling during this phase and appear exquisitely vulnerable to the effects of stress (Spear, 2000). Alterations in hypothalamic-pituitary-adrenal (HPA) axis function have also been noted during this developmental window (McCormick & Mathews, 2007; McCormick & Mathews, 2010; Romeo et al., 2006; Spear, 2000)

Animal studies investigating the impact of stressor exposure during juvenility have demonstrated unique outcomes in both brain and endocrine function. Interestingly, adolescent rats show a differential HPA axis response to stressors relative to adults, including a heightened or prolonged adrenocorticotrophin releasing hormone and corticosterone release and a failure to habituate to repeated stressors (McCormick & Mathews, 2010). However, these responses appear to vary based on the type of stressor employed and some observations are sex dependent (McCormick & Mathews, 2010). Stressors have also been demonstrated to have a unique behavioral impact when applied in juvenility (McCormick & Green, 2013). For example, juvenile rats tend to display an increased in locomotor and exploratory behavior in novel environments while adults display a more

anxiogenic response (Doremus, Varlinskaya, & Spear, 2004; Stansfield & Kirstein, 2006). Moreover, juvenile stressors have been shown to have protracted behavioral and neurochemical ramifications that extend into adulthood. Previous exposure to non-social stress in juvenility has been associated with anxiety-like behavior in both the open field (Horovitz et al., 2012; Ilin & Richter-Levin, 2009) and EPM (Ilin & Richter-Levin, 2009; Jacobson-Pick & Richter-Levin, 2010; Jacobson-Pick & Richter-Levin, 2012), as well as impairments in avoidance learning (Horovitz et al., 2014; Ilin & Richter-Levin, 2009). Results detailing the long-term implications of non-social stressors on HPA axis functioning have been mixed with some studies reporting elevations in basal corticosterone (Ilin & Richter-Levin, 2009), anther others reporting no differences in basal corticosterone (Jacobson-Pick & Richter-Levin, 2010; Mackay et al., 2014).

Social stressors may be of particular relevance to the period of adolescence. It has been argued that the large majority of stressors encountered in adolescence include a social component (Buwalda et al., 2011). Indeed the increased prevalence of social stress in adolescence highlights the importance of employing animal models that best emulate the target of study. In this regard, the resident intruder paradigm has been proposed to be an ethologically relevant model to study the impact of social stress in rodents (Miczek, 1979; Rygula et al., 2005; Wood et al., 2010). In this paradigm, a rat (intruder) is introduced into the home cage of a larger, more aggressive rat (resident), followed by repeated threatening encounters (e.g. no direct contact with resident). This paradigm has also been referred to as social defeat, as the intruder rat is often subject to aggressive behaviors from the resident designed to demonstrate dominance (Miczek, 1979). In adult male rodents, social defeat is associated with altered HPA axis activity, increased depressive-like behaviors, promotion of anhedonia, decreased social interaction, increased propensity to use psychostimulants, and increased risk for cardio-vascular disease and

other health related pathologies (Becker et al., 2008; Buwalda et al., 1999; Chang et al., 2009; Covington, III et al., 2005; Heinrichs et al., 1992; Miczek et al., 2004; Rygula et al., 2005; Von Frijtag et al., 2000; Wood et al., 2010; Wood et al., 2009). However, there is a paucity of studies focusing on the impact of the resident intruder applied in juvenility. The few studies to date have associated juvenile social defeat with decreased social interaction and exploration behavior in adulthood (Burke et al., 2011; Vidal et al., 2007; Watt et al., 2009), increased anxiety (Weathington et al., 2012), and increases in proactive coping behavior (Bingham et al., 2011). While other have suggested no enduring long-term impacts (Bourke & Neigh, 2011; Buwalda et al., 2013; Hayashida et al., 2010).

While the impact of social defeat in juvenility has begun to be elucidated, even less is known about the potential stress mitigating effects of highly palatable foods in juvenility. Among adult rats, access to a palatable food has been proposed to protect against learned helplessness after an inescapable foot shock (Dess, 1992), reduce sympathetic responses to a stressor (Buwalda et al., 2001; Christiansen et al., 2011; Fachin et al., 2008; Kant & Bauman, 1993; la Fleur et al., 2005; Pecoraro et al., 2004; Ulrich-Lai et al., 2007; Ulrich-Lai et al., 2010; Ulrich-Lai et al., 2011; Young, 2000), and diminish HPA axis activity (Foster et al., 2009; Levin, 1996; Strack et al., 1997; Zeeni et al., 2012). However, this proposed self-medication with food (Dallman et al., 2005) comes at a price, including negative metabolic consequences and obesity (for review see Patterson & Abizaid, 2013). Nevertheless, there is some evidence to suggest that access to a palatable diet may have mitigating effects on the impact of early life stress. For example, the provision of a palatable high fat diet has been shown to normalize the negative long-term behavioral (Maniam & Morris, 2010a; Maniam & Morris, 2010b) and neuroendocrine (Lee et al., 2014) consequences of maternal separation. As responses to stressors appear differ between

adolescent and adult rats (Avital & Richter-Levin, 2005; Bingham et al., 2011; Romeo, 2010), characterization of the use of palatable foods as a means of coping with stress during adolescence may be particularly relevant given the climate of increased obesity across all age groups.

In this regard, the present study sought to further characterize the long term impact of juvenile social defeat on behavioral measures of depressive- and anxiety-like behavior, as well as basal corticosterone. A secondary objective was to determine the potential mitigating effect of a highly palatable diet on the aforementioned measures. The metabolic consequences of access to a palatable diet are also explored.

Materials and Methods

Animals. Fifty-six 21 day old male Sprague Dawley rats (50-55g) were used as intruders. Sixteen Long Evans retired breeders (600g) were used as residents following resident screening from a pool of thirty-five residents. A separate group of ten 21 day old male Sprague Dawley rats were used to screen residents. All rats were obtained from Charles River (Quebec, Canada) and singly housed in standard plastic cages with bedding at a room temperature of 22±1°C on a 12 hour light-dark cycle (lights on at 0700hrs). Weight gain was measured on a weekly basis. All procedures met the guidelines established by the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Ottawa Institute of Mental Health Research.

Experimental design. An overview of the stress procedure and behavioral testing schedule is presented in Figure 5. Upon arrival, intruders rats were randomly assigned to one of four conditions: (1) Chow + No Stress; (2) Chow + Stress; (3) Palatable + No Stress; and (4) Palatable + Stress. All animals underwent all behavioral tests, and glucose tolerance testing.

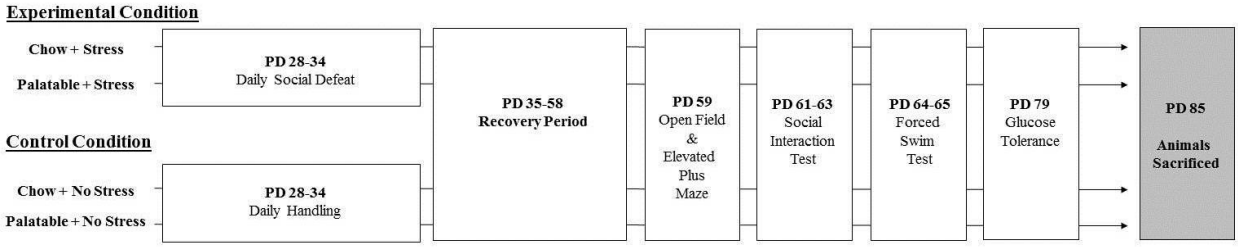

*Figure 5.*Summary of study design. Animals assigned to the social defeat condition were exposed to a different resident rat per day from PD 28-34 while control animals received daily handling. All animals were tested in the indicated behavioral paradigms.

Diet. All rats were given *ad libitum* access to standard laboratory chow (3.4 kcal/g, 4.5% fat, 18.1% protein, 57.3% carbohydrate, Charles River Rodent Diet 5075, Agribrand Purina Canada, Woodstock, Ontario, Canada) and rats assigned to the palatable food condition were given 4 hours of access to a 45%Kcal fat pellet (4.7 Kcal/g, 23.2% fat, 17.3% protein, 47.6% carbohydrates, TD.08811, Harlan Laboratories, Madison, Wisconsin, USA). Palatable food was given daily at 8:00 and removed at 12:00 and intake was measured by subtracting the remaining amount from the initial weight. Every 7 days, food consumption was measured by subtracting the weight of the food from the food weight 24-hours earlier. Total caloric intake was calculated by multiplying the energy content (Kcal/g) of individual foods by the amount consumed. Calories from the palatable diet were calculated by multiplying the amount of food consumed by the energy content of the diet. Food intake data was collected from PD 21 to PD60.

Resident screening. One week prior to the arrival of study animals, resident rats were screened for aggressive behavior. Screening took place over 5 days where each resident received one exposure to an intruder animal that was introduced to the resident's home cage. Sessions

lasted a maximum of 15 minutes. Intruders were removed from the cage of the resident once they had been defeated. Latency to attack the intruder, number of fights and latency to defeat was recorded. Following screening residents were ranked based on the average number of fights with the intruder per day and latency to attack. The top 16 residents with the shortest latency to attack and highest number of fights were selected for use in the study.

Social defeat paradigm. The resident-intruder stress was performed as described by Bingham and colleagues (2011). Briefly, rats in the defeat condition (intruders) were introduced into the home cage of a different resident daily for 7 consecutive days (PDs 28-34). Intruders and residents were allowed to interact for a maximum of 15 min and then a plastic holed divider was used to separate the rats. Interactions were terminated before the 15 min mark if the intruder was defeated or if the intruder was attacked 5 times. Following separation, the intruder remained in the resident's cage for the remainder of the time allotted for the test session (60 min including the 15 min interaction). This prevented further physical contact between the resident and intruder while maintaining olfactory and visual cues and thus maintaining the psychosocial nature of the stress (Rygula et al., 2005). Residents were used twice per day.

Behavior during resident-intruder interactions. Resident-intruder sessions were analyzed for several behaviors, including: Latency to separation; latency to submission; total time spent in submissive postures (sum of full and side submission); time spent in full submission (intruder pinned on back) and side submission (intruder pinned on side), time spent fighting; and social behavior of intruders towards residents (aggression, over/under, sniffing) (Gardner, Thrivikraman, Lightman, Plotsky, & Lowry, 2005; Paul et al., 2011). Measures of the time spent engaging in each of the behaviors were adjusted for the time each intruder spent with a given resident by taking the ratio of the behavior by the time spent with the resident (e.g. time spent in

submission/ latency to separation). All behaviors are represented as the average time over the 7 defeat sessions.

Behavioral testing. Behavioral testing was conducted under low illumination (30 - 40 lux) between 10:00-13:00 daily following a 1 hr. period during which rats habituated to the testing room. Behavior was monitored via a video camera mounted above the arena.

Open field. On PD 59, prior to elevated plus maze (EPM) testing, rats were placed in the center of the open field (OF) arena and its behavior monitored for 5 min. The arena consisted of a square Plexiglas arena measuring 60 x 60 cm with 30 cm high walls. Using lines, the floor was divided up into 16 squares (5 x 5cm). The total number of squares crossed was recorded as an index for general locomotor activity (Correa, Arizzi, Betz, Mingote, & Salamone, 2003). The amount of time spent in the centre and periphery of the maze was recorded and increased time spent in the periphery of the maze is indicative of an anxiogenic response (Walsh & Cummins, 1976).

Elevated plus maze. On PD 59, rats were placed on the center platform of the EPM (facing a closed arm), immediately after OF testing. The EPM consisted of two open arms (50 x 10 cm) and two perpendicularly situated arms enclosed by 40 cm high walls, elevated approximately 66 cm above the floor. Behaviors scored during the 5 min test included time spent on the open and closed arms, risk assessment behavior (unprotected head dips; head protruding over the edge of an open arm) and number of entries into the closed arms. Time in the open arms and unprotected head dips are validated measures of reduced anxiety-like behavior (Carobrez & Bertoglio, 2005). The number of entries into the closed arms of the maze are viewed as a measure of spontaneous locomotor activity (Walf & Frye, 2007).

Social interaction. The social interaction test was conducted over a total duration of three days. On habituation day 1 (PD 61), rats were matched to a partner from the same Diet x Stress condition (but from a different cage) on the basis of body weight, such that members of a pair did not differ by >10g. Paired rats were then placed in the arena (60 x 60 cm; 30cm high walls) together for five minutes. On habituation day 2 (PD 62), all rats were individually placed in the arena for a period of three min. On test day (PD 63), the same pairs of rats used on habituation day 1 were placed in the arena and social behaviors of both rats were observed for 7 min. Total time spent in social interaction (including sniffing, climbing over each other, following, allogrooming, and play fighting) was recorded and each individual analyzed separately. Decreases in social interaction in rodents may be reflective of an anxiogenic profile (File & Seth, 2003).

Forced swim test. The forced swim test is a widely used behavioral despair paradigm used to evaluate the effectiveness of antidepressant drugs (Porsolt et al., 1977; Porsolt et al., 1977; Schifter, 1991). The forced swim arena consisted of a clear Plexiglas cylinder (20 x 45 cm, water depth 30 cm, temperature 25 °C; Stoelting Co., Wood Dale, Illinois). A habituation session (PD 64: 15 min) was performed twenty-four hours prior to the test session. On test day (PD 65) the animal was returned to the same cylinder for 5 minutes. The time spent immobile was recorded during the test session. Immobility was defined as an animal floating in the water without struggling, and only engaging in movements to keep the head above water (Slattery & Cryan, 2012). Increases in immobility are thought to reflect behavioral despair (Cryan et al., 2002; Lucki, 1997). Therefore reduced immobility is used to assess the antidepressant properties of a given compound (Cryan et al., 2005).

Glucose tolerance. On PD 79, following 12 hr. of fasting, all rats were given an intraperitoneal injection (IP) of a 0.75g/mL dextrose solution (dose: 1.75g/kg). Blood glucose was measured by applying a drop of blood (via tail venipuncture) onto a test strip, then taking a reading with a blood glucose meter (Accu-Chek Aviva Nano, Roche Diagnostics, Mannheim, Germany). Levels were assessed immediately before the injection, and 15, 30, 60 and 120 min post-injection.

Tissue Processing. On PD 85 animals were sacrificed by rapid decapitation and carcasses were stored at -80°C until fat analysis. Trunk blood was immediately collected in tubes containing EDTA (70 μM), placed on ice and spun in a refrigerated centrifuge (4°C, 3000 rpm, 15 min), after which plasma was isolated and frozen at -80 °C until analysis.

Plasma Corticosterone. Corticosterone levels were measured from isolated plasma using a commercial RIA kit (MPbiomedicals, Solon, OH) according to manufacturer instructions. These methods have been detailed previously (Merali et al., 2009). The inter- and intra-assay variability was 10.5% and 8.79%, respectively.

Adiposity. Frozen carcasses were thawed to room temperature and weighed. Quantitative magnetic resonance imaging (EchoMRI, Echo Medical Systems, Houston, TX) was used to determine the body composition (fat, lean mass, and water) of the carcasses.

Statistical analysis. Data obtained from the OF, EPM, social interaction, FST, plasma corticosterone, and adiposity were analyzed by 2-way analysis of variance (ANOVAs) where Diet (2 levels; chow vs. palatable food) and Stress (2 levels; stress vs no stress) were the between group factors. Body weight was analyzed using 3-way repeated-measures ANOVAs, where Diet (2 levels) and Stress (2 levels) were the between group variables and Time (9 points) the repeated within group variable. Total caloric intake, and glucose tolerance results were analyzed

using 3-way repeated-measures ANOVAs, where Diet (2 levels) and Stress (2 levels) were the between group variables and Time (5 points) the repeated within group variable. Calories from the palatable diet was analyzed using 2-way repeated-measures ANOVAs, where Stress (2 levels) was the between group variable and Time (5 points) the repeated within group variable.

Subsequent follow-up comparisons were conducted using *t* tests with a Bonferroni correction to maintain the alpha level at .05. For some measures a priori predictions that access to the palatable diet would alter the impact of the stressor were made. Analyses of the simple effects for interactions and a priori predictions were conducted irrespective of whether the F value for an interaction reached significance (Winer, 1962) , especially when visual inspection of graphed results suggested that a significant difference may exist. Data points ±3 standard deviations from calculated means were considered as outliers and not included in statistical analysis (Taylor, 1997). Some rats were removed from the statistical analysis of variable due to missing data, thus the N and df associated with these measures vary across outcomes.

Results

Behavior during defeat sessions. Overall, rats with access to the palatable diet spent significantly more time with the residents during the defeat sessions compared to Chow fed rats as measured by latency to separation, F (1,24) = 7.56, p = .011; Figure 6A. One-way ANOVAs also revealed significant effects of Diet for some intruder behaviors during the defeat sessions after correcting for the amount of time spent with the residents. In this regard, rats with access to the palatable diet spent significantly less time fighting with the resident, F(1,24) = 5.77, p = .024, as well less total time in submissive postures, F(1,24) 18.61, p < .001. They also spent less time in full submission, F(1,24) = 7.75, p = . = .010 and side submission, F(1,24) = 12.06, p = .002 (Figure 6B). No significant differences were observed in time spent engaging in social behavior

initiated by the intruder (Figure 6C) including: total social behaviors, $F(1,24) = .102$, $p = .75$; moving over and under the resident $F(1,24) = 1.56$, $p = .22$; sniffing, $F(1,24) = .72$, $p = .41$; following, $F(1,24) = 1.29$, $p = .27$; and aggression towards the resident, $F(1,24) = 2.80$, $p = .11$.

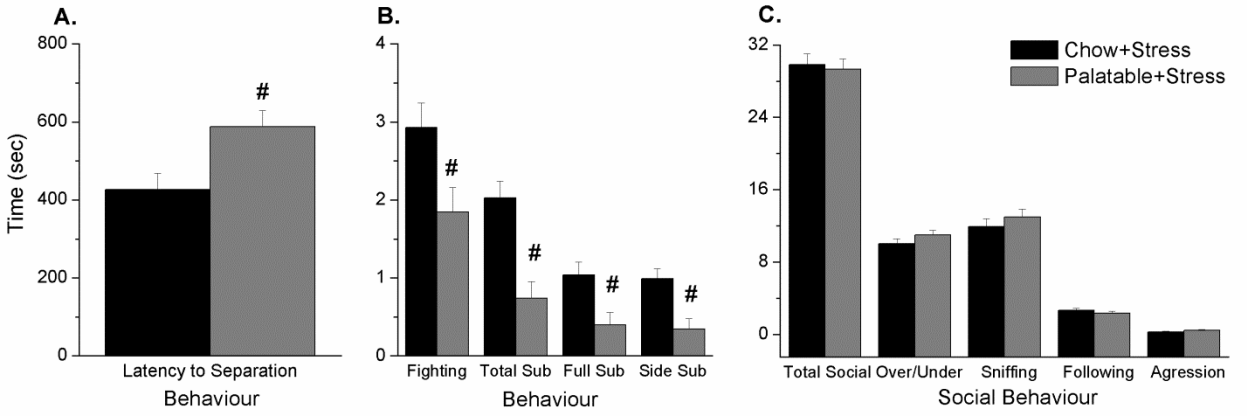

Figure 6. Behavior of intruders during defeat sessions. In general, rats with access to the palatable diet spent more time with the residents. (A). After correcting for the time spent with the residents, defeated rats with access to the palatable diet spent less time in submissive postures, as well as less time fighting with the resident (B). No differences in intruder initiated social behaviors were observed (C). Bars represent mean ± SEM. # Significant effect of Diet at $p < .05$.

Impact of social defeat and diet on body weight and feeding behavior. Repeated measures ANOVA revealed a significant Diet x Stress x Time interaction for body weight, $F_{Stress\ x\ Diet\ x\ Time}(4,416) = 8.61$, $p = .001$; Figure 7A. Follow-up simple effect analyses revealed that defeated Chow-fed rats weighed significantly less than their controls in the three weeks following exposure to social defeat, whereas no weight differences were observed between defeated rats with access to palatable food compared to their controls at any time points.

No significant effect of Diet x Time, $F_{Diet\ x\ Time}$ (4,208) = 1.79, p = .132, and Stress x Time, $F_{Stress\ x\ Time}$ (4,208) = 1.95, p = .103, was observed in the total amount of calories consumed. The interaction between Diet x Stress x Time was also non-significant $F_{Stress\ x\ Diet\ x\ Time}$ (4,208) = 1.54, p = .191; Figure 7B. Repeated measures ANOVA revealed a significant Stress x Time effect on the consumption of the palatable diet, $F_{Stress\ x\ Time}$ (4,104) = 3.22, p = .015; Figure 7C; however follow-up comparisons of the individual time points revealed no significant differences in intake.

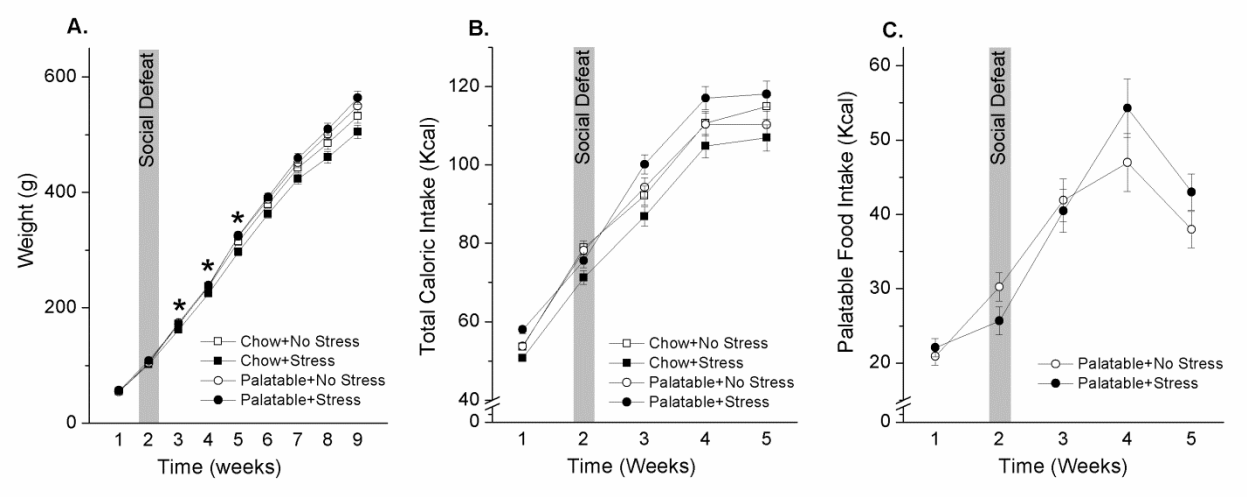

Figure 7. Impact of social defeat and diet on body weight, caloric intake and preference for the palatable diet. A significant decrease in the rate of weight gain among the Chow + Stress group was observed in the 3 weeks following social defeat. Points represent mean ± SEM. * Significant effect of Stress at $p < .05$.

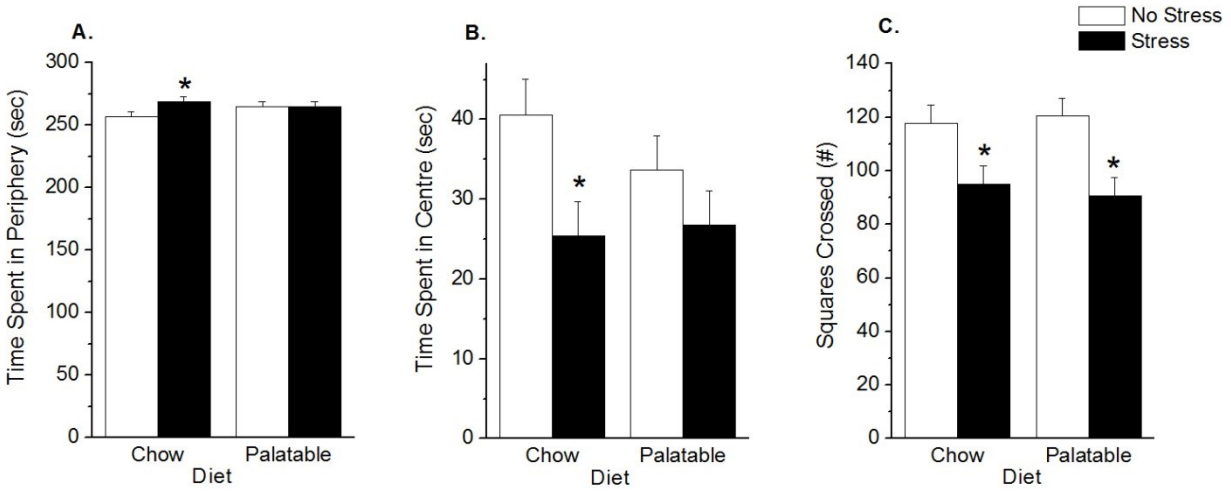

Figure 8. Impact of social defeat and diet on behavior in the open field test. Previous exposure to social defeat was associated with an anxiogenic profile amongst rats with access to chow only. Bars represent mean ± SEM. * Significant effect of Stress at $p < .05$;

Impact of social defeat and diet on behavioral markers of depression and anxiety.

Previous exposure to social defeat in juvenility was associated with an anxiogenic profile in the open field test. A significant Stress effect was observed in the time spent in the center of the maze, F_{Stress} (1,51) = 6.60, p = .013; Figure 8B and the number of squares crossed, F_{Stress} (1,51) = 15.11, p < .001; Figure 8C. The main effect of Diet and interaction between Stress x Diet was non-significant for both time spent in the centre of the maze, F_{Diet}(1,51) = .409, p = .525 and $F_{Stress\ x\ Diet}$(1,51) = .938, p = .337, and number of squares crossed, F_{Diet}(1,51) = .013, p = .909 and $F_{Stress\ x\ Diet}$(1,51) = .282, p = .597. No significant effect of Stress, F_{Stress} (1,51) = 2.312, p = .134, and Diet, F_{Diet} (1,51) = .274, p = .603 were observed in time spent in the periphery of the arena. The interaction between Stress x Diet was also non-significant $F_{Stress\ x\ Diet}$ (1,51) = 1.97, p = .17; Figure 8A. Post hoc comparisons were completed based on an a priori hypothesis that a

significant Stress x Diet interaction would be presented for all variables. Simple effects analysis revealed that rats with access to chow only spent significantly less time in the centre of the maze and more time in the periphery of the maze relative to their controls. Whereas decreased locomotor behavior was observed in all defeated rats regardless of diet.

Diet and exposure to juvenile social defeat did not impact behavior in the elevated plus maze (Table 2). Two-way ANOVA indicated no significant main effect of Stress, F_{Stress} (1,48) = .550, p = .462, and Diet, F_{Diet} (1,48) = .348, p = .558, on time spent in the open arm; the interaction between Stress and Diet was non-significant $F_{Stress\ x\ Diet}$ (1,48) = .060, p = .807. Two-way ANOVA indicated no significant main effect of Stress, F_{Stress} (1,48) = .335, p = .566, and Diet, F_{Diet} (1,48) = .013, p = .909, on time spent in the closed arm; the interaction between Stress and Diet was non-significant $F_{Stress\ x\ Diet}$ (1,48) = .116, p = .735. Two-way ANOVA indicated no significant main effect of Stress, F_{Stress} (1,48) = 2.12, p = .152, and Diet, F_{Diet} (1,48) = .009, p = .926, on entries in the closed arm; the interaction between Stress and Diet was non-significant $F_{Stress\ x\ Diet}$ (1,48) = .090, p = .725. Two-way ANOVA indicated no significant main effect of Stress, F_{Stress} (1,48) = .816, p = .371, and Diet, F_{Diet} (1,48) = .160, p = .212, on the number of risk assessments made; the interaction between Stress and Diet was non-significant, $F_{Stress\ x\ Diet}$ (1,48) = .222, p = .640.

A significant Stress effect was observed in the total time spent engaging in social behaviors in the social interaction test, F_{Stress}(1,51) = 7.94, p = .007; Figure 9A . The main effect for Diet, F_{Diet} (1,51) = .443, p = .509, and interaction between Stress x Diet, $F_{Stress\ x\ Diet}$ (1,51) = .843, p = .363, were non-significant. Follow-up comparisons were completed based on an a priori hypothesis that a significant Stress x Diet interaction would be present. Simple effect analyses

revealed that defeated rats with access to Chow only displayed the significant increase in total social behavior relative to their diet-matched controls.

Table 2

Impact of juvenile social defeat and diet on behavior in the EPM and FST.

	Chow+No Stress	Chow+Stress	Palatable+No Stress	Palatable+Stress
EPM				
Time spent in open arms	92.92 ± 17.56	84.16 ± 16.92	86.84 ± 19.09	69.41 ± 16.92
Time spent in closed arms	141.21 ± 19.29	136.59 ± 18.57	145.58 ± 20.97	127.77 ± 18.59
Entries into closed arm	8.00 ± 0.69	6.79 ± 0.66	7.23 ± 0.75	6.93 ± .66
Number of risk assessments	3.23 ± 0.86	2.86 ± 0.83	2.55 ± 0.94	1.36 ± 0.83
FST				
Time spent immobile	42.36 ± 13.10	34.79 ± 12.62	52.67 ± 13.10	55.16 ± 12.62

Mean ± SEM
EPM = elevated plus maze
FST = forced swim test

Individual social behaviours and locomotor behaviour are depicted in Figure 9B-C. Exposure to social defeat also significantly impacted time spent allogrooming, F_{Stress} (1,51) = 17.92, p < .001,and time the rats spent moving over and under each other, F_{Stress} (1,51) = 5.94, p = .018. The main effect of Diet and interaction between Stress x Diet were non-significant for both allogrooming, F_{Diet} (1,51) = 1.33, p = .254 and $F_{Stress\ x\ Diet}$ (1,51) = .463, p = .499, and time the rats spent moving over and under each other, F_{Diet} (1,51) = 1.04, p = .321 and $F_{Stress\ x\ Diet}$ (1,51) = .802, p = .375. No significant differences were observed in all other behaviours, including time spent sniffing [F_{Stress} (1,51) = 1.17, p = .284, F_{Diet} (1,51) = 3.48, p = .068 , and

$F_{Stress\ x\ Diet}$ (1,51) = 3.41, p = .070], following [F_{Stress} (1,51) = 1.25, p = .268, F_{Diet} (1,51) = .342, p = .561 , and $F_{Stress\ x\ Diet}$ (1,51) = 010, p = .920], play fighting [F_{Stress} (1,51) = 2.83, p = .099, F_{Diet} (1,51) = 2.91, p = .094 , and $F_{Stress\ x\ Diet}$ (1,51) = 2.00, p = .16], and locomotor activity [F_{Stress} (1,51) = .597, p = .443, F_{Diet} (1,51) < .001, p = .998 , and $F_{Stress\ x\ Diet}$ (1,51) = 1.55, p = .219]. Again, follow-up comparisons were completed based on an a priori hypothesis that significant Stress x Diet interactions would be present for these individual behaviors. Simple effect analyses revealed that all defeated rats displayed increased allogrooming relative to their controls. However, significant increases, time spent sniffing their partner, and time spent moving over and under their partner were observed amongst defeated rats with access to Chow only. Defeated rats with access to the palatable diet were observed to display increased play fighting behavior relative to their diet matched controls.

Exposure to social defeat and access to a palatable diet did not impact behavior in the FST (Table 2). Two-way ANOVA revealed no significant main effect of Stress, F_{Stress} (1,50) = .039, p = .844, and Diet, F_{Diet} (1,51) = 1.42, p = .238, on the time spent immobile. The interaction between Stress and Diet was also non-significant, $F_{Stress\ x\ Diet}$ (1,50) = .152, p = .698.

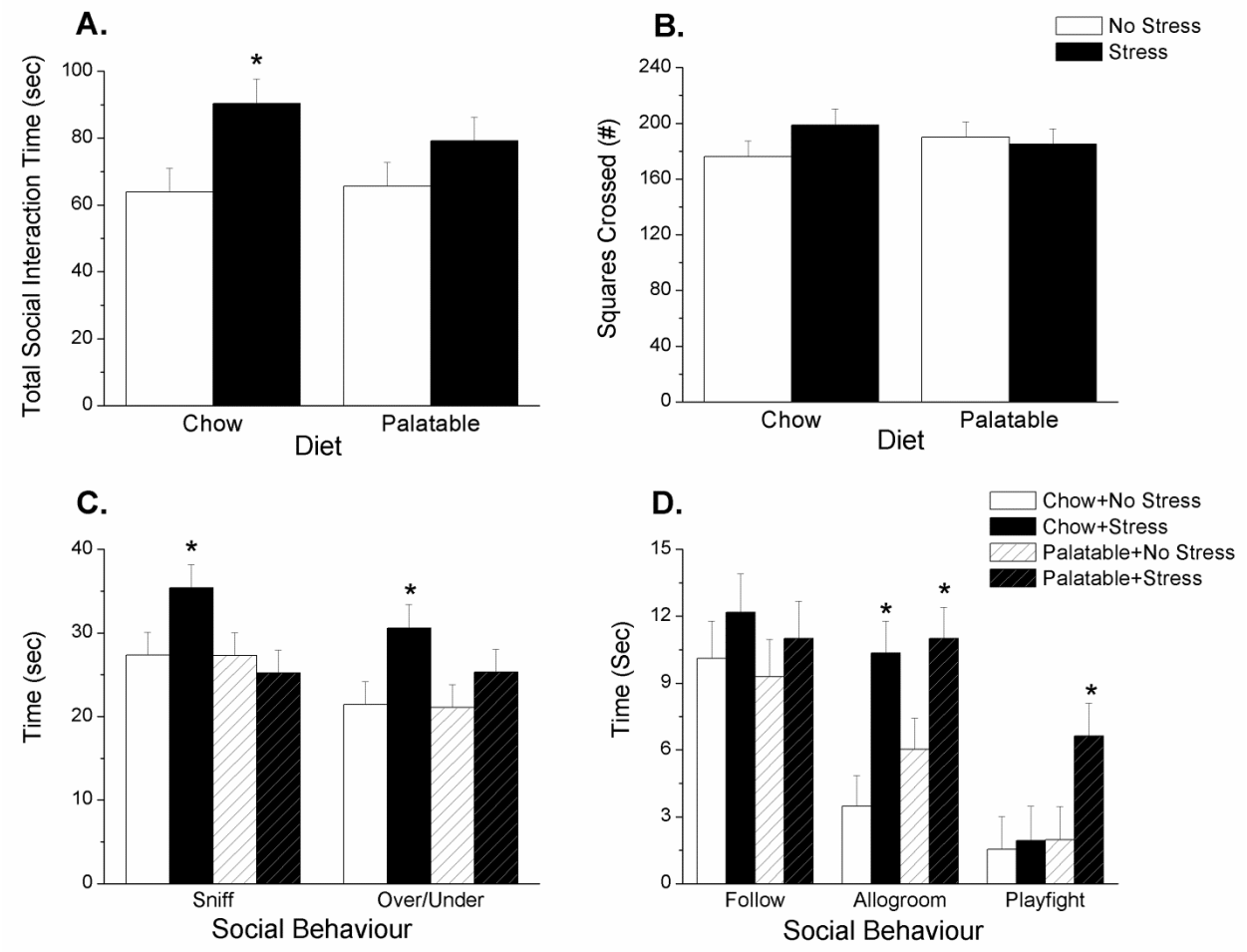

Figure 9. Impact of social defeat and diet on behavior in the social interaction test. In general defeated rats with access to Chow only displayed an increased in sociability relative to their controls. Bars represent mean ± SEM. * Significant effect of Stress at $p < .05$

Impact of social defeat and diet on endocrine and metabolic indices. Results of endocrine and metabolic indices are summarized in Table 3. Two-way ANOVA revealed no significant main effect of Diet, $F_{Diet}(1,51) = .417$, $p = .521$, and Stress, $F_{Stress}(1,51) = 1.34$, $p = .253$, on basal plasma corticosterone levels. The interaction between Stress and Diet was non-significant, $F_{Stress \times Diet}(1,51) = .118$, $p = .733$, Table 3. Similarly, 3-way ANOVA indicated no

significant effects of Diet x Time, $F_{Diet\ x\ Time}(4,208) = .422$, $p = .793$Stress x Time, $F_{Stress\ x\ Time}(4,208) = 1.80$, $p = .130$, and interaction between Stress x Diet x Time, $F_{Stress\ x\ Diet\ x\ Time}(4,208) = .40$, $p = .81$, were observed on the ability to tolerate a glucose load.. Two-way ANOVA indicated no significant main effect of Stress, $F_{Stress}(1, 50) = .1.58$, $p = .215$, and Diet, $F_{Diet}(1, 50) = .703$, $p = .406$, on total body fat percentage; the interaction between Stress and Diet was non-significant $F_{Stress\ x\ Diet}(1, 50) = .231$, $p = .633$.

Table 3

Impact of juvenile social defeat and diet endocrine and metabolic indices.

	Chow+No Stress	Chow+Stress	Palatable+No Stress	Palatable+Stress
Plasma corticosterone (ng/mL)	44.55 ± 13.89	65.58 ± 13.89	58.41 ± 14.41	69.81 ± 13.89
Glucose Tolerance [a]				
0 min	5.43 ± 0.27	5.27 ± 0.27	5.67 ± 0.27	6.14 ± 0.27
30 min	23.17 ± 2.10	24.74 ± 2.10	21.35 ± 2.10	25.50 ± 2.10
60 min	21.16 ± 1.73	21.81 ± 1.73	19.81 ± 1.73	24.19 ± 1.73
90 min	15.56 ± 1.21	14.93 ± 1.21	13.66 ± 1.21	15.17 ± 1.21
120 min	9.09 ± 0.55	8.73 ± 0.55	8.05 ± 0.55	8.52 ± 0.55
Adiposity				
Body fat percentage	25.50 ± 0.86	24.37 ± 0.86	24.01 ± 0.86	23.71 ± 0.86

min = minutes
[a] Blood glucose level expressed as mmol/L
All data represented as Mean ± SEM

Discussion

The present study sought to further characterize the long term impact of juvenile social defeat on behavioral measures of depressive- and anxiety-like behavior, as well as HPA axis response to a novel stressor. Although there are many studies to date elucidating the impact of social defeat in adulthood, less is known about exposure during juvenility. To date the literature

is mixed, with some studies reporting long-term differences in social and anxiety-like behavior (Burke et al., 2011; Vidal et al., 2007; Vidal et al., 2011a; Watt et al., 2009; Weathington et al., 2012) and others reporting no differences (Bourke & Neigh, 2011; Buwalda et al., 2013; Hayashida et al., 2010). Results of the present study would appear to suggest that exposure to juvenile defeat may have had some persisting effects into adulthood.

While previous exposure to social defeat was associated with a mild anxiogenic profile in the open field test, there were no apparent differences in the EPM, which is consistent with previous results (Bourke & Neigh, 2011; Buwalda et al., 2013; Hayashida et al., 2010). Open field results are inconsistent with previously reported results (Bingham et al., 2011), where no immediate differences in behavior on the open field were observed. While a similar social defeat methodology was employed, follow-up behavioral testing occurred much later in the present study (PD 35-37 vs PD 59-65 in the present study) making direct comparison difficult. In addition, as with studies investigating adult resident intruder interactions, the methodology employed in juvenility varies greatly. Indeed apparent long-term effects of juvenile social defeat may depend on the developmental period during which it is employed. For example, exposure to social defeat between PD 35-40 is associated with increased exploratory behavior in adulthood (Burke et al., 2011; Watt et al., 2009) whereas present results suggest that exposure during an earlier developmental window (PD 28-34) resulted in the opposite effect. It is also possible that prolonged exposure to defeat may engender different results. For example, male and female juvenile rats exposed to social defeat from PD 28 to 39 displayed a pattern of increased anxiety- and depressive-like behavior in adulthood (Weathington et al., 2012). Exposure to juvenile social defeat also did not appear to impact immobility in the FST when rats were tested in adulthood. As with the EPM, previous work has focused on the immediate impact of social defeat as

opposed to the longer-term consequences. Immediate effects in juvenility appear to be mixed and may be dependent on the age of exposure (Bingham et al., 2011), as well as the chronicity of the paradigm (Bourke et al., 2014; Hayashida et al., 2010). There is evidence to suggest that the long-term effects of adolescent social defeat on behavior in the FST may be sexually dimorphic, as female rats demonstrate decreased activity in the FST in adulthood (Bourke & Neigh, 2011; Ver Hoeve, Kelly, Luz, Ghanshani, & Bhatnagar, 2013; Weathington et al., 2012), whereas males do not (Bourke & Neigh, 2011; Weathington et al., 2012). In general, female rats appear to be more sensitive to the long-term impact of social defeat, which also includes sensitization of HPA axis response to a novel stressor and increased anxiety-like behavior in the EPM relative to males (Weathington et al., 2012).

Housing conditions are also highly relevant in this paradigm as social housing has been shown to act as a buffer against the negative impact of social defeat (Ruis et al., 1999). For example, when rats that have been defeated in juvenility are housed with a non-defeated cage mate no differences in open field behaviors are observed in adulthood; however, alterations in home cage behavior are observed, where defeated rats demonstrated an increase in the initiation of play behavior, as well as assumption of submissive postures during play (Buwalda et al., 2013). Similar findings have been previously reported among subordinate rats (Pellis & Pellis, 2007). In the present study, rats were individually housed in order to avoid the potential attenuating effects of social housing, as well as confounding effects of the hierarchical positions in the home cage (Bingham et al., 2011). Matched controls were also singly-housed, which suggests that the observed differences in the open field may be attributed to juvenile defeat.

In adulthood, previous exposure to juvenile social defeat was associated with an increase in sociability which was most pronounced among rats with access to chow only. These findings

appear to be in contrast to previous reports of reduced social behaviors following juvenile social defeat (Vidal et al., 2007; Vidal et al., 2011b; Watt et al., 2009). One explanation for the observed differences in the present study may be the fact that rats were housed individually. Social play during adolescence is an important component for the development of adult social behavior (Einon & Morgan, 1977; Pellis & Pellis, 2007). Social isolation or deprivation during juvenility is associated with behavioral and social deficits in adulthood (Pellis & Pellis, 2007). For example, when isolated juveniles are given 1-hour access to a juvenile peer, the observed social deficits in adulthood are absent compared to isolates with no social access whatsoever (Einon, Morgan, & Kibbler, 1978). Interestingly, housing juvenile rats with a non-playful adult female does not alleviate the social deficits in adulthood, suggesting that it is the play exposure that is key (Einon et al., 1978). This is further supported by an experiment where rats were pair housed but separated by wire mesh barriers allowing for olfactory cues, as well as huddling, but not play contact. Consistent with the non-playful female, this type of housing condition did not offset adult social deficiencies (Pellis, Field, & Whishaw, 1999). In the present study, defeated juvenile rats had some form of social contact during this critical stage in development relative to control rats. It is possible that exposure to defeat may have, in some way, contributed to the development of adult social behaviors by somewhat emulating social play, thus making defeated rats somewhat more socially competent relative to their controls. It is noteworthy that, when tested in adulthood, all rats were matched with test partners from the same experimental conditions, which may have impacted the social dynamic. Additionally, there is some evidence to suggest that previous exposure to social defeat may influence the reward associated with future social encounters. In this regard Syrian hamsters exposed to aggressive social encounters demonstrated a preference for social interaction when tested in the condition place preference

two months after initial conditioning trials, whereas hamsters with no previous social experience demonstrated no preference (Gil, Nguyen, McDonald, & Albers, 2013). This preference was observed regardless of whether or not the animal had dominant or subordinate status in the initial encounter, suggesting that exposure to any form of social interaction may be rewarding.

The observed anxiety-like behavior in the open field was most prominent amongst rats with access to chow only suggesting that access to the palatable diet may have had some minor mitigating effects. We have previously reported stress-buffering effects of palatable foods on anxiety-like behavior in adulthood following exposure to a non-social juvenile stressor (Mackay et al., 2014). While results across studies are not directly comparable given that different stressors were employed, they add to the growing evidence that access to a palatable diet may alter behavioral expressions of adult anxiety-like behavior following juvenile stressor exposure. Access to the palatable diet also appeared to be protective against defeat-induced reduction in weight gain among Chow fed rats. Decreased rate of weight gain is a well-established consequence of stressor exposure in rats (Marti, Gavalda, Jolin, & Armario, 1996). These results appear to be consistent with our previously reported results where access to a palatable diet appeared to provide some protection against the stress-induced reduction in weight gain following exposure to non-social stressor in juvenility (Mackay et al., 2014).

Interestingly, access to the palatable diet appeared to impact behavior during resident-intruder interactions. In this regard, juvenile rats with access to the palatable diet spent more time with the resident rat as evidenced by the increase latency to separation. After controlling for the effects of time, rats with access to the palatable diet also spent less time in submissive postures; however, no differences in general social behaviors were observed. The present results would seem to suggest that access to the palatable diet may have imparted some degree of resilience

within the interactions, which is somewhat supported by the more prominent anxiety-like behavior amongst rats with access to Chow only. Out results raises the question of the potential temporal onset of the stress-buffering effects of the palatable diet. Consumption of the palatable diet during defeat may have attenuated the relative severity of the stressor resulting in less functional impact, suggesting that stressor differences in adulthood may be attributable to initial stress-buffering effects of the palatable diet as opposed to long-term diet exposure. Further studies investigating behavioral outcomes in juvenility may provide additional information regarding the temporal onset of the potential stress-buffering effects of a palatable diet.

Consistent with some previous reports (Burke et al., 2011; Vidal et al., 2007; Vidal et al., 2011a; Watt et al., 2009; Weathington et al., 2012), results of the present investigation would seem to suggest that exposure to social defeat during juvenility may have a long-term impact on anxiety-like behavior in adulthood, as measured by the open field test. The observation that defeated rats with access to the palatable diet displayed a potential resilience during resident-intruder interactions has, to our knowledge, has not been previously reported. While we observed some modest stress-buffering effects of the palatable diet in the long-term, it is possible that the diet may have also mitigated the short term-effects of social defeat; however, this remains to be characterized.

Disclosure

All of the authors declare that they have no direct or indirect conflicts of interests of a financial nature relevant to the present work.

Acknowledgements

This project was funded by an operating grant from the Canadian Institute for Health Research (CIHR) awarded to Zul Merali, and by a CIHR doctoral Canada Graduate Scholarship

awarded to J. Christine MacKay. Technical contributions of Kaitlin Baenziger and Samantha Graitson are gratefully acknowledged.

Preface to Chapter 4:

What is the short and long term impact of a non-social episodic stress during adolescence? Does access to a palatable high-fat, high-sugar diet mitigate the consequences of juvenile stress exposure?

As stated previously, the relationship between stress, eating behavior and obesity is germane to the period of adolescence given the recent increases in the prevalence of obesity in this population. While the resident-intruder paradigm offers an ethologically relevant method to investigating the role of a social stressor in this phenomenon, is it somewhat fraught with methodological challenges. Specifically, in the experiment presented in Chapter 2 priming aggressive behavior towards the intruder in the residents was particularly challenging. While we made attempts to only select residents that would reliably attack intruders during the screening phase, it was difficult to maintain consistency across all resident-intruder interactions. Similar challenges have also been noted (Bingham et al., 2011). We observed some modest stress-buffering effects of the palatable diet in Chapter 2; however, juvenile social defeat on its own was solely associated with a mild anxiogenic profile in the open field test. The results of the experiment presented in Chapter 2, as well as its accompanying methodological challenges influenced a decision to move towards the use of a non-social juvenile stress paradigm.

Jacobson-Pick and Ritcher-Levin (2010) have demonstrated significant short and long-term negative neurochemical and behavioral consequences using a 3-day model of different stressors during juvenility. In this paradigm, juvenile rats are exposed to 3 consecutive days of stress with a different stressor per day (forced swimming, elevated platform, and restraint) from PD 27-29. Across studies, juvenile stress has resulted in significant reductions in exploratory

behavior, reduced exploratory behavior and reduced exploration of high-risk areas in the elevated plus maze in adulthood and impaired avoidance learning in adulthood (Ilin & Richter-Levin, 2009; Jacobson-Pick & Richter-Levin, 2010; Tsoory et al., 2007; Tsoory & Richter-Levin, 2006) as well as neurobiological consequences (Avital et al., 2006; Jacobson-Pick & Richter-Levin, 2010; Jacobson-Pick & Richter-Levin, 2012). Given that this stress paradigm has been shown to have long-term behavioral consequences, the present experiment set out to evaluate whether or not access to a palatable diet may mitigate the potential long-term negative consequences.

Several individuals were involved in the design and implementation of this experiment, as well as the preparation and editing of the manuscript. Mr. Jonathan James assisted with data collection and the processing of endocrine and metabolic markers. Mr. Christian Cayer assisted with the analysis of data collected from behavioral assays. Dr. Hymie Anisman provided conceptual input and manuscript edits. Dr. Pamela Kent assisted with data analysis and manuscript edits. Dr. Zul Merali provided guidance and input during the conceptualization and design of the study, as well as on-going feedback throughout data collection, analysis and manuscript preparation. Conceptual input regarding the results of the present experiment was provided by all authors. The author of this dissertation is the first author of the present manuscript and was involved in all aspects of the design and implementation of the experiments, as well as analysis of results and preparation of the manuscript.

Chapter 4: Protracted effects of juvenile stressor exposure are mitigated by access to palatable food.

Authors

J. Christine MacKay

Jonathan Stewart James

Christian Cayer

Pamela Kent

Hymie Anisman

Zul Merali

Citation: MacKay JC, James JS, Cayer C, Kent P, Anisman H, et al. (2014) Protracted Effects of Juvenile Stressor Exposure Are Mitigated by Access to Palatable Food. *PLoS ONE* 9(5): e96573. doi:10.1371/journal.pone.0096573

Abstract

Stressor experiences during the juvenile period may increase vulnerability to anxiety and depressive-like symptoms in adulthood. Stressors may also promote palatable feeding, possibly reflecting a form of self-medication. The current study investigated the short- and long-term consequences of a stressor applied during the juvenile period on anxiety- and depressive-like behavior measured by the elevated plus maze (EPM), social interaction and forced swim test (FST). Furthermore, the effects of stress on caloric intake, preference for a palatable food and indices of metabolic syndrome and obesity were assessed. Male Wistar rats exposed to 3 consecutive days of variable stressors on postnatal days (PD) 27-29, displayed elevated anxiety-like behaviors as adults, which could be attenuated by consumption of a palatable high-fat diet. However, consumption of a palatable food in response to a stressor appeared to contribute to increased adiposity.

Keywords

Juvenile Stress; Anxiety; Obesity; High Fat Diet; Palatable Food

Introduction

Adolescent and childhood obesity has become a worldwide epidemic to the extent that globally, approximately 200 million school aged children can be classified as either overweight or obese (International Obesity Task Force, 2010). Further, childhood and adolescent obesity is a strong predictor of adult obesity (Singh et al., 2008; Spriijt-Metz, 2011; Whitaker et al., 1997), and have been associated with adverse long-term health outcomes such as, hypercholesterolemia, insulin resistance (Shaibi & Goran, 2008), hypertension (Freedman et al., 1999), type-2 diabetes (Pinhas-Hamiel & Zeitler, 1996), non-alcoholic fatty liver disease (Cruz et al., 2005) and various cancers (Calle & Kaaks, 2004; Calle & Thun, 2004).

Recent increases in the prevalence and incidence of obesity have been attributed to the interplay of a variety of different factors, such as genetics, the family environment, levels of physical activity, advertising, and sedentary behaviors (Hills et al., 2011). Of particular interest is the role of stress in the development and maintenance of obesity, as the level of daily stressors individuals have been experiencing continues to increase (Coccurello et al., 2009a; Hill & Peters, 1998; Torres & Nowson, 2007a). In this regard, a stressor-induced preference for palatable foods (especially those with a high fat and/or sugar content) has been documented in both animals and humans (Dallman, 2010; Gibson, 2006; O'Connor et al., 2008; Zellner et al., 2006). This preference was proposed to serve as a form of self-medication to protect against the adverse effects of stress (Dallman et al., 2005). Access to a palatable food has been shown to protect against behavioral disturbances elicited by inescapable foot shock (Dess, 1992), reduce sympathetic responses to a stressor (Buwalda et al., 2001; Christiansen et al., 2011; Fachin et al., 2008; Kant & Bauman, 1993; la Fleur et al., 2005; Pecoraro et al., 2004; Ulrich-Lai et al., 2007; Ulrich-Lai et al., 2010; Ulrich-Lai et al., 2011; Young, 2000), and diminish hypothalamic-

pituitary adrenal (HPA) axis activity (Foster et al., 2009; Levin, 1996; Strack et al., 1997; Zeeni et al., 2012). Consumption of palatable foods may also limit some of the long-term negative effects of an early life stressor (maternal separation) (Maniam & Morris, 2010a).

Although self-medication with a palatable food can have some beneficial effects on psychological functioning, it may be a counterproductive long-term stress coping strategy. The excess calories gained from consumption of a high fat and/or sugar diet leads to an increase in adipose tissue (de Ferranti & Mozaffarian, 2008), which secretes several hormones and signaling factors (Bastard et al., 2006; Kriketos et al., 2004; Woods & Seeley, 2000) involved in the regulation of food intake (Korner & Leibel, 2003; Woods & Seeley, 2000). Increases of adipose tissue and the resulting endocrinological consequences have been implicated in atherosclerosis (de Ferranti & Mozaffarian, 2008), elevated plasma levels of inflammatory cytokines (Bastard et al., 2006), and elevated concentrations of free fatty acids, which can contribute to reduced muscle glucose uptake (Randle, 1998) and the development of insulin resistance, which has been directly linked to type 2 diabetes, cardiovascular disease, and cancer (DeFronzo & Ferrannini, 1991; Jee et al., 2005).

The relationship between stress, eating behavior and obesity during adolescence may be of particular significance given the increased prevalence of obesity in this population. Indeed, in both humans and rodents, the juvenile phase represents a critical period in development during which there is substantial cerebral development and reorganization as well as altered HPA axis function (Bingham et al., 2011; McCormick & Mathews, 2010). It has been suggested that this major biological transition period renders adolescents more sensitive to the effects of stressors and the subsequent development of stressor-related psychopathologies (Avital & Richter-Levin, 2005). Animal studies have shown unique effects of stressors on HPA activity during adolescence as pre-

pubertal rats exhibited higher or prolonged adrenocorticotrophin releasing hormone and corticosterone release compared to adults (McCormick & Mathews, 2010). However, these effects were sex dependent and also varied with the stressor employed (McCormick & Mathews, 2010). Adolescence is further characterized as a unique stage in brain development, as regions related to emotional and learning process, such as the prefrontal cortex, hippocampus, and the amygdala, all undergo substantial remodeling during this phase and have also been proposed to be exquisitely sensitive to the effects of stress (Spear, 2000). Importantly, these regions have also been implicated in the homeostatic mechanisms underlying energy balance and feeding behaviors (Dallman, 2010; Spear, 2000).

The present study was conducted to further characterize the short- and long-term consequences of stressor exposure during the juvenile period (PD 27-29) on behavioral indices of depression and anxiety using a modified version of the juvenile stress protocol developed previously (Jacobson-Pick & Richter-Levin, 2010). A second objective was to determine the effects of juvenile stressors on feeding behavior and preference for a palatable food. In this regard, we aimed to elucidate whether the consumption of a palatable food during adolescence could attenuate the long-term behavioral consequences of stressor exposure. A final objective of this study was to investigate the long term consequences of stress-induced palatable feeding on indices of metabolic syndrome and obesity.

Materials and Methods

Animals. Ninety-six 21 day old male Wistar rats were obtained from Charles River (Quebec, Canada). Rats were double housed until PD 30 or 60 in standard plastic cages (37cm x 26 cm x 18cm) with bedding at a room temperature of 22±1°C on a 12 hour light-dark cycle (lights on at 0700hr). On PD 30 or 60 all animals were singly housed in order to prepare for

social interaction testing. Animals remained singly housed for the remainder of the experiment. Weight gain was measured on PDs 25, 30, 40, 50, 60, 70. All procedures met the guidelines established by the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Ottawa Institute of Mental Health Research.

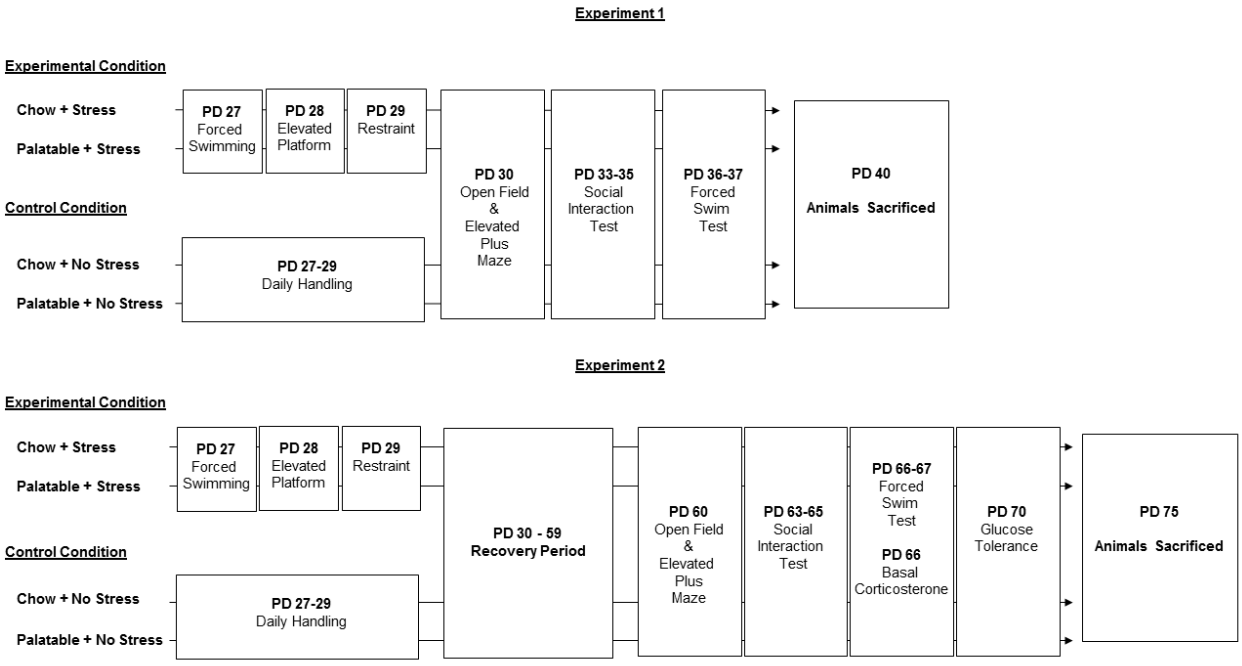

*Figure 10.*Summary of study design. Animals assigned to stress condition were exposed to a different stressor per day from PD 27-29 while control animals received daily handling. All animals were tested in the indicated behavioral paradigms as described in each experiment.

Experiments. An overview of the stress procedure and behavioral testing schedule is presented in Figure 10. Upon arrival, rats were randomly assigned to four conditions: (1) Chow + No Stress; (2) Chow + Stress; (3) Palatable + No Stress; and (4) Palatable + Stress. Forty rats were used in Experiment 1 (juvenile testing, PD-30-37) and fifty-six rats were used in Experiment 2 (adult testing, PD 60-67). Animals in both experiments underwent all behavioral

tests. Weight gain, caloric intake, comfort preference, glucose tolerance, plasma corticosterone and adiposity were only collected in Experiment 2.

Diet. All rats were given free access to standard laboratory chow (3.4 kcal/g, 4.5% fat, 18.1% protein, 57.3% carbohydrate, Charles River Rodent Diet 5075, Agribrand Purina Canada, Woodstock, Ontario, Canada) and rats assigned to the palatable food condition were given limited daily access to a 45%Kcal Fat diet (4.7 Kcal/g, 23.2% fat, 17.3% protein, 47.6% carbohydrates, TD.08811, Harlan Laboratories, Madison, Wisconsin, USA). Palatable food was given daily at 8:00 and removed at 10:00 and intake was measured. Every three days, chow consumption was measured by subtracting the weight of the chow in the cage from its weight 24-hours earlier. Total caloric intake was calculated by multiplying the energy content (Kcal/g) of individual diets by the amount consumed and summing them. Preference for palatable food was calculated by taking the ratio of calories of palatable food consumed over total calories consumed. Food intake data was collected from PD 21 to PD60. As rats were housed in pairs, equal food intake by cage mates was assumed.

Juvenile stress paradigm. The juvenile stress procedure used was a modification of the 3 day procedure described by Jacobson-Pick and Ritcher-Levin (2010). This procedure comprised 3 consecutive days of exposure to a different stressor per day throughout PD27-29.

PD 27 - Forced swimming. Rats were individually placed in a circular water basin (diameter 48cm; height 42cm; water depth 29 cm and a temperature of 22±2°C) for 10 min, during which they swam or floated continuously.

PD 28 – Elevated platform. Rats were individually placed on a small elevated platform (12cm x 12cm; 70cm elevation from water level) for three separate 30 min sessions. Platforms stood within a basin filled with water for the animals' protection should they fall off. During the

intersession interval of 90 min rats were returned to their home cage. Animals that fell during the testing session were immediately returned to the platform for the remainder of the test session.

PD 29 – Restraint. Rats were placed for 30 min in a plastic restraining bag that prevented side-to-side movement and limited forward-backward movement. The plastic bag had a hole at the end closest to the rat's nose to allow for ventilation.

Behavioral testing. Behavioral testing was conducted under low illumination (30 - 40 lux) between 10:00-13:00 daily following a 1 hr period during which rats habituated to the testing room. Behavior was monitored via a video camera mounted above the arena.

Open field. On PD 30 or 60, prior to elevated plus maze (EPM) testing, rats were placed in the center of the open field (OF) arena and its behavior monitored for 5 min. The arena consisted of a square Plexiglas arena measuring 60 x 60 cm with 30 cm high walls. Using lines, the floor was divided up into 16 squares (5 x 5cm). The total number of squares crossed was recorded as an index for general locomotor activity (Correa et al., 2003).

Elevated plus maze. On PD 30 or 60, rats were placed on the center platform of the EPM (facing a closed arm), immediately after OF testing. The EPM consisted of two open arms (50 x 10 cm) and two perpendicularly situated arms enclosed by 40 cm high walls, elevated approximately 66 cm above the floor. Behavior scored during the 5 min test included time spent on the open and closed arms, risk assessment behavior (unprotected head dips; head protruding over the edge of an open arm), and number of entries into the closed arm. Time in the open arms and unprotected head dips are validated measures of reduced anxiety-like behavior (Carobrez & Bertoglio, 2005). The number of entries into the closed arms of the maze are viewed as a measure of spontaneous locomotor activity (Walf & Frye, 2007).

Social interaction. The social interaction test was conducted over a total duration of three days. On PD 30 or 60 rats were individually housed 2 hours after the completion of EPM testing in order to increase the level of social interaction (File & Seth, 2003). On habituation day 1 (PD 33 or 63), rats were matched to a partner from the same Diet x Stress condition (but from a different cage) on the basis of body weight, such that members of a pair did not differ by >10g. Paired rats were placed in the arena (60 x 60 cm; 30cm high walls) together for five minutes. On habituation day 2 (PD 34 or 64), all rats were individually placed in the arena for a period of 3 min. On test day (PD 35 or 65), the same pairs of rats used on habituation day 1 were placed in the test chamber and the behavior of both rats was observed for 7 min. Total time spent in social interaction (including sniffing, climbing over each other, following, allogrooming, and play fighting,) was recorded. Decreases in social interaction in is reflective of an anxiogenic profile (File & Seth, 2003).

Forced swim test. The forced swim test is a widely used behavioral despair paradigm used to evaluate the effectiveness of antidepressant drugs (Porsolt et al., 1978; Porsolt et al., 1977; Schiller et al., 1992). The forced swim arena consisted of a clear Plexiglas cylinder (20 x 45 cm, water depth 30 cm, temperature 25 °C; Stoelting Co., Wood Dale, Illinois). A habituation session (PD 36 or 66: 15 min) was performed twenty-four hours prior to the test session. On test day (PD 37 or 67) the animal was returned to the same cylinder for 5 minutes. The time spent immobile was recorded during the test session. Immobility was defined as an animal floating in the water without limb movements. Increases in immobility are thought to reflect behavioral despair (Cryan et al., 2002; Lucki, 1997). Therefore reduced immobility is used to assess the antidepressant properties of a given compound (Cryan et al., 2005).

Basal corticosterone levels. On PD 66, prior to the FST training session, rats from Experiment 2 were moved in their home cages to the testing room and allowed to rest for a minimum of one hour. Rats had been singly housed on PD 60 and blood samples were collected from rats individually using tail venipuncture. The time elapsed from retrieving the rat from their home cage to the depositing the blood sample on the filter paper took approximately 2 min per rat. Blood droplets were deposited onto 903 Protein Saver filter paper (GE Healthcare Bio-Sciences Corp, MA, USA), allowed to dry at room temperature then stored at -20°C.

Collected blood droplets were analyzed for whole blood corticosterone levels using commercial radioimmunoassay (RIA) kits (MP biomedical, CA, USA). Two days prior to the RIA procedure, blood was eluted from the filter paper by placing one 3 mm punch (per time point) of filter paper in a 12 x 75 culture tube containing 200 µL Dulbecco's Phosphate Buffered Saline (Sigma, item D-5773) w/0.1% gelatine, covered with parafilm in a fridge at 4°C. On the day of the RIA procedure, culture tubes containing the samples were placed on an orbital shaker for 1 hour at room temp. Corticosterone levels were the determined from the eluted blood sample using the commercial RIA kits as per the manufacturer's instructions. The inter- and intra-assay variability was 7.3% and 7.4%, respectively.

Glucose tolerance. On PD 70, following 12hr of fasting, rats in Experiment 2 were given an intraperitoneal injection (IP) of a 0.75g/mL dextrose solution (dose: 1.75g/kg). Blood glucose was measured by applying a drop of blood (via tail venipuncture) onto a test strip, then taking a reading with a blood glucose meter (Accu-Chek Aviva Nano, Roche Diagnostics, Mannheim, Germany). Levels were assessed immediately before the injection, and 15, 30, 60 and 120 min post-injection.

Adiposity. On PD 75, carcasses of animals in Experiment 2 were collected following sacrifice then stored at -20°C. At a later date the carcasses were thawed at 4°C and fat pads hand dissected and weighed. The fat pads collected included: mesenteric, retroperitoneal, subcutaneous inguinal white fat, and inter-scapular brown fat.

Statistical analysis. Data obtained from the OF, EPM, social interaction, FST, basal corticosterone, and adiposity were analyzed by 2-way analysis of variance (ANOVAs) where Diet (2 levels; chow vs. palatable food) and Stress (2 levels; stress vs no stress) were the between group factors. Body weight was analyzed using 3-way repeated-measures ANOVAs, where Diet (2 levels) and Stress (2 levels) were the between group variables and Time (6 points) the repeated within group variable. Total caloric intake was analyzed using 3-way repeated-measures ANOVAs, where Diet (2 levels) and Stress (2 levels) were the between group variables and Time (10 points) the repeated within group variable. Calories from the palatable diet was analyzed using 2-way repeated-measures ANOVAs, where Stress (2 levels) was the between group variable and Time (10 points) the repeated within group variable. Glucose tolerance results were analyzed using 3-way repeated-measures ANOVAs, where Diet (2 levels) and Stress (2 levels) were the between group variables and Time (5 points) the repeated within group variable.

Subsequent follow-up comparisons were conducted using *t* tests with a Bonferroni correction to maintain the alpha level at .05. For some measures a priori predictions that access to the palatable diet would alter the impact of the stressor were made. Analyses of the simple effects for interactions and a priori predictions were conducted irrespective of whether the F value for an interaction reached significance (Winer, 1962), especially when visual inspection of graphed results suggested that a significant difference may exist. Data points ±3 standard deviations from calculated means were considered as outliers and not included in statistical

analysis (Taylor, 1997). Some rats were removed from the statistical analysis of variable due to missing data, thus the N and df associated with these measures vary across outcomes.

Results

Effect of stress and diet on weight and food intake. Juvenile stressor exposure and diet had a significant effect on body weight. Repeated measures ANOVA revealed a significant Stress, $F(5,255) = 889.72$, $p < .001$, and Diet effect, $F(5,255) = 1125.12$, $p < .001$;Figure 11A and B. Follow-up comparisons were completed based on an a priori hypothesis that a significant interaction would be present. Simple effects analysis revealed that previously stressed chow fed rats weighed significantly less than controls at PDs 40, 60, and 70. Similarly, among previously stressed rats with access to the palatable diet, a significant decrease in weight relative to the palatable control group was observed on PDs 30, 40, and 50. In general, rats with access to the palatable diet weighed more than Chow fed rats.

Repeated measures ANOVA revealed a significant effect of Diet $F(9,207) = 2.83$, $p = .026$ on total caloric intake (Figure 11C). In general, rats with access to the palatable diet consumed more calories. No significant effect of Stress on preference for palatable food was observed, $F(9,99) = 1.895$, $p = .061$; Figure 11D.

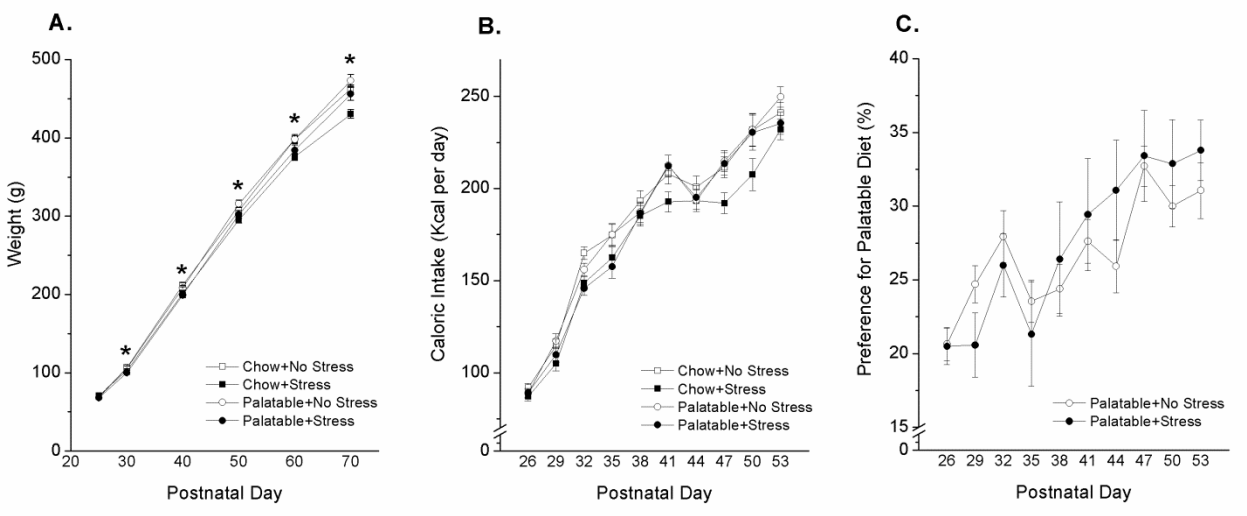

Figure 11. Effect of stress and diet on weight and food intake. Initially, both stress groups gained weight (A and B) at the slower rate relative to their controls; however, this reduction in the rate of weight gain persisted past PD50 in the Chow+Stress group only. Overall, rats with access to the palatable diet consumed more calories relative to chow-fed rats (C). No significant difference in preference for the palatable diet was observed (D). Points represent mean ± SEM. * Significant effect of Stress at $p < .05$.

Effect of stress and diet on anxiety- and depressive-like behavior. Locomotor behavior across groups was comparable in both Experiment 1 and 2. No significant effects of Stress or Diet were found regarding number of squares crossed in the open field at both the juvenile and adult time point. . Means ± standard error of the mean (SEM) for the juvenile time point were as follows: Chow + No Stress 123.10±14.26; Chow + Stress 118.00±10.25; Palatable + No Stress 140.10±11.36 and Palatable + Stress 128.10±3.85. Means ±SEM for the adult time point were as follows: Chow + No Stress 104.19±6.41; Chow + Stress 98.57±7.81; Palatable + No Stress 103.85±6.16 and Palatable + Stress 93.86±4.02.

In the EPM (Figure 12, A-D), a significant main effect for Diet on the amount of time spent on the open arms was observed in Experiment 1, $F(1,35) = 4.717$, $p = .037$. In general, juvenile rats with access to the palatable diet spent more time on the open arms of the maze. No significant differences in time spent in the closed arm, and number of risk assessments was observed. No significant effects of Stress or Diet were found regarding entries into the closed arm suggesting that locomotor activity across groups was comparable.

In Experiment 2, a significant main effect for Stress was observed in the EPM (Figure 12, E-H) for time spent in the open arms, $F(1,50) = 12.271$, $p = .001$ and time spent in the closed arms, $F(1,50) = 13.55$, $p = .001$, suggesting that the juvenile stressor induced long-term anxiety-like behaviors. Follow-up simple effects comparisons on time spent on the open and closed arms were completed based on an a priori hypothesis that a significant interaction would be present. Interestingly, previously stressed rats with access to chow spent significantly less time in the open arm, and spent more time in the closed arms of the maze compared to their controls. No significant differences were observed in terms of the number of risk assessments made and number of entries into the closed arm entries, again suggesting that locomotor activity was comparable across groups.

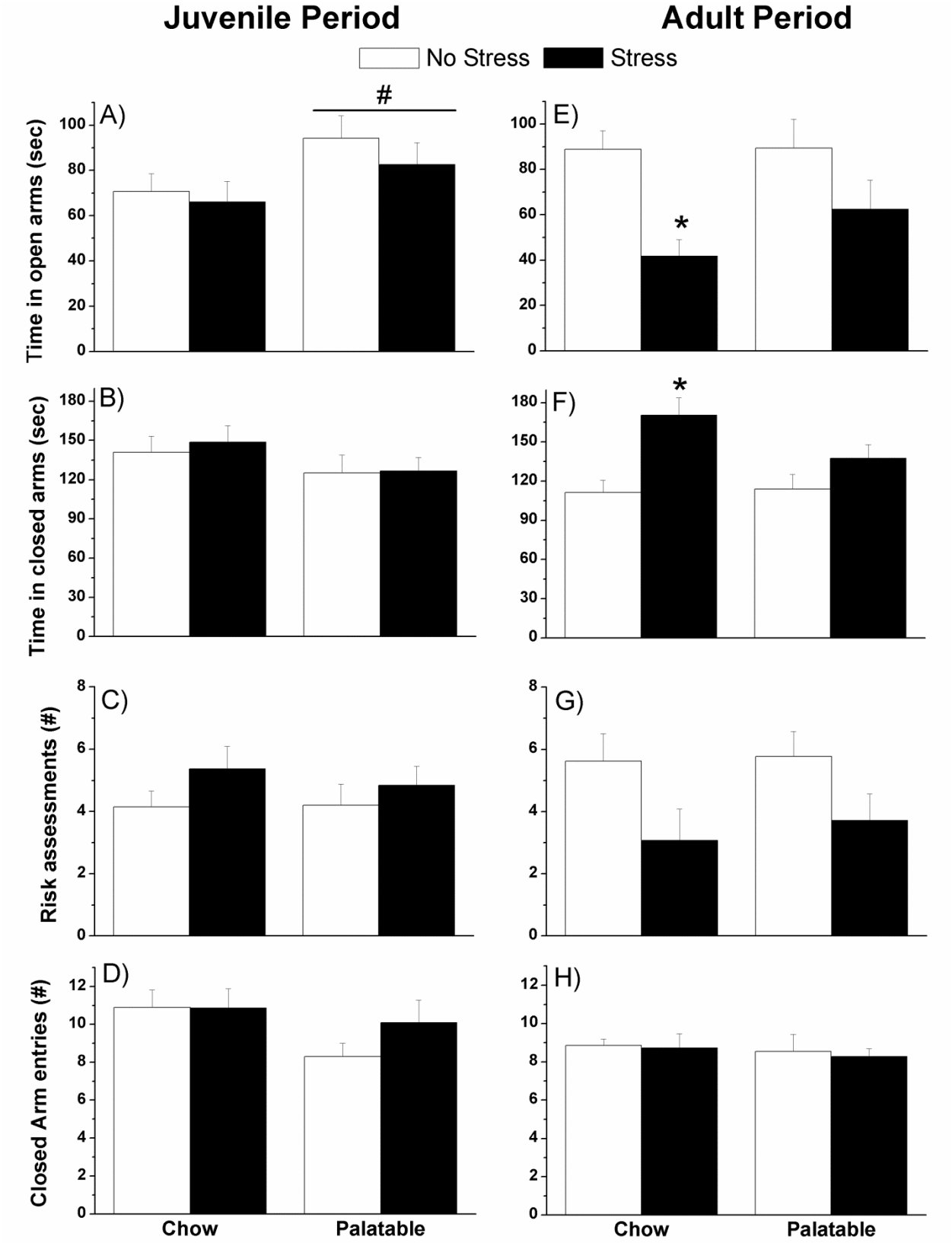
Juvenile Period
Adult Period
No Stress
Stress
A)
B)
C)
D)
E)
F)
G)
H)
Time in open arms (sec)
Time in closed arms (sec)
Risk assessments (#)
Closed Arm entries (#)
#
*
*
Chow
Palatable
Chow
Palatable

Figure 12. Anxiety-like behavior in the elevated plus maze. A significant effect of the palatable diet was observed in the juvenile time point. In general, rats with access to the palatable diet spent more time in the open arms of the maze (A). In adulthood, previously stressed chow fed rats (E) spent less time in the open arms, and (F) more time in the closed arms compared to chow fed controls. No significant difference across groups was observed regarding number of (G) risk assessments and closed arms entries (H), suggesting no significant differences in locomotor activity. Bars represent mean ± SEM. * Significant effect of Stress at $p < .05$; # Significant effect of Diet at $p < .05$.

In the social interaction test, no significant differences in total time spent engaging in active social behaviors was observed in juvenile rats (Figure 13, A-B). In regard to individual social behaviors, a significant Diet effect was observed in time spent play fighting $F(1,35) = 5.250$, $p = .028$) as rats with access to the palatable diet engaged in more play fighting.

In Experiment 2, juvenile stress induced a long-term increase in anxiety-like behavior measured in the social interaction test (Figure 13, C-D). A significant interaction between Stress and Diet was found with regard to total time spent engaged in social behaviors $F(1,51) = 9.085$, $p = .004$). The follow-up simple effects analyses confirmed that previously stressed rats with access to chow spent significantly less time engaged in active social interaction compared to non-stressed controls. This was also observed in individual social behaviors, including following, and moving over/under one another and time spent sniffing on another. No effect of stress was observed in the palatable food group.

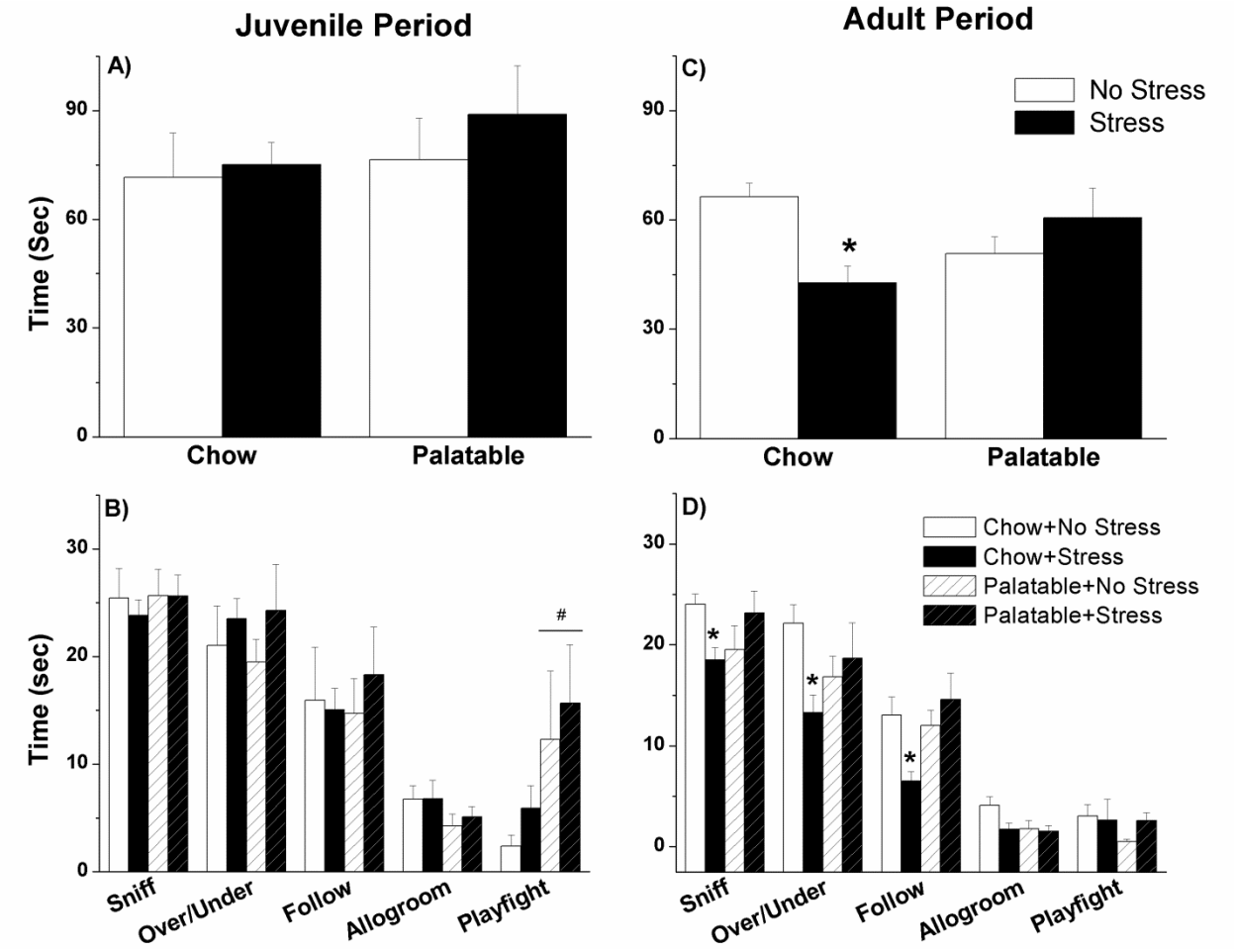

Figure 13. Social behavior in the social interaction test. No significant differences in total time engaged in social behaviors were observed in the juvenile phase (A); however, in general rats with access to the palatable diet engaged in more play fighting (B). In adulthood, previously stressed chow fed rats spent less total time engaged in social behaviors compared to chow-fed controls and previously stressed rats with access to the palatable diet (C). In regards to specific behaviors, chow fed rats exposed to the juvenile stress spent less time sniffing, following and moving over/under one another (D) relative to their controls. The difference between stress groups was significant for following behavior as well (D). Bars represent mean ± SEM. * Significant effect of Stress at $p < .05$; # Significant effect of Diet at $p < .05$.

For both the juvenile and adult time points, no significant effects of Stress or Diet were found regarding time spent immobile in the FST. Mean ±SEM time spent immobile for the juvenile time point was as follows: Chow + No Stress 5.208±1.20; Chow + Stress 3.805±1.20; Palatable + No Stress 4.060±1.27 and Palatable + Stress 3.298±1.20. Mean ±SEM time spent immobile for the adult time point was as follows: Chow + No Stress 21.29±3.96; Chow + Stress 20.89±3.96; Palatable + No Stress 27.52±4.11 and Palatable + Stress 19.47±4.11.

Effect of stress and diet on endocrine and metabolic indices. A significant Diet effect was observed in basal corticosterone levels measured on PD66, $F(1,50) = 10.56$, $p = .002$, wherein rats with access to the palatable diet displayed significantly reduced basal corticosterone (Figure 14A).

Repeated measures ANOVA revealed a significant Time x Stress interaction for blood glucose levels in response to a glucose challenge, $F(4,200) = 2.621$, $p = .036$; Figure 14B. Although previously stressed rats with access to the palatable diet tended to show increased blood glucose relative to their controls follow-up simple effects analysis did not reach significance.

In general all rats with access to the palatable diet displayed an increase in adiposity (Figure 14C). A significant Diet effect was observed in the weight of retroperitoneal and subcutaneous inguinal fat pads, $F(1,52) = 10.93$ $p = .002$ and $F(1,52) = 10.93$ $p = .002$, respectively. Two-way ANOVA of mesenteric fat pad weight revealed a significant Diet x Stress interaction, $F(1,52) = 4.75$ $p = .034$). Follow-up simple effects analysis revealed that previously stressed rats with access to chow displayed significant decreases in mesenteric fat relative to the chow-fed control group. No significant differences in inter-scapular brown fat were observed $F(1,52) = 1.69$ $p = .20$.

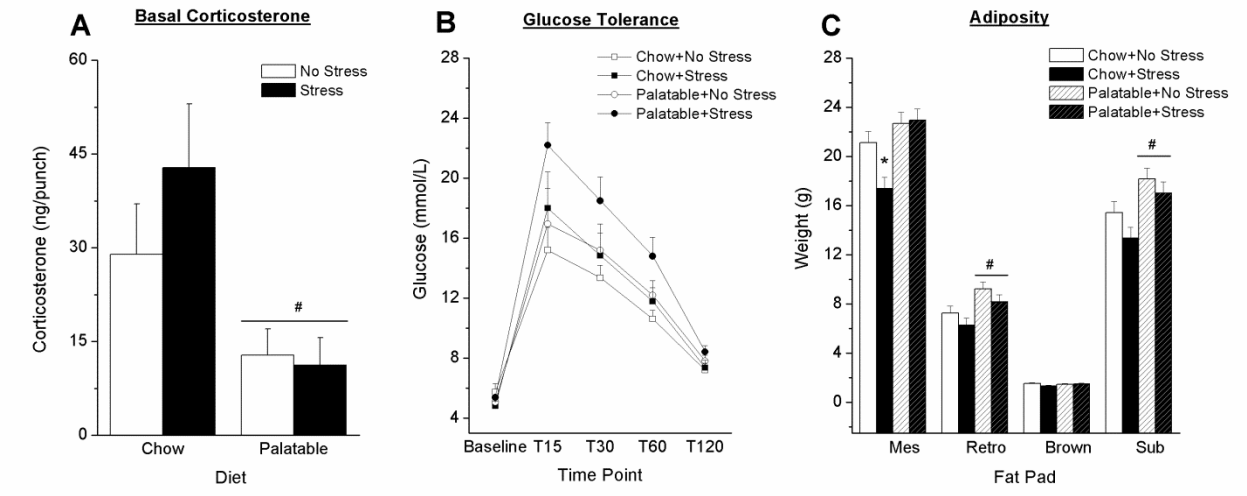

Figure 14. Effect of stress and diet on endocrine and metabolic indices. Rats with access to palatable food displayed lower plasma levels of corticosterone compared to chow fed rats on PD 66 (A). Blood glucose levels over time following an IP. injection of a 0.75g/mL dextrose solution did not differ significantly across groups (B). Rats with access to the palatable diet displayed an increased in retroperitoneal and inguinal subcutaneous fat (C). A decreased weight of the mesenteric fat pad was observed in the Chow+Stress condition relative to non-stressed controls. Mes = Mesenteric fat pad. Retro = retroperitoneal fat pad. Brown = Inter-scapular brown fat. Sub = Subcutaneous inguinal white fat pad. Data represents mean ± SEM. * Significant effect of Stress at $p < .05$; # Significant effect of Diet at $p < .05$.

Discussion

The present study investigated the short- and long-term consequences of unpredictable physical stress applied during the juvenile period on subsequent behavioral markers of depression and anxiety and indices of metabolic syndrome and obesity. No short-term effects of juvenile stress on behavioral markers of anxiety- and depressive-like behaviors were observed in

the open field, EPM and social interaction test. In contrast, juvenile stress resulted in decreased exploration of high-risk areas of the EPM in adulthood, which is consistent with results reported by Jacobson-Pick and Ritcher-Levin (Jacobson-Pick & Richter-Levin, 2010). Reduced social exploration was also observed in the present study, as rats previously exposed to juvenile stress exhibited less social behavior compared to controls. Decreased social behavior in adulthood following juvenile stress has been previously reported in both mice (Jacobson-Pick, Audet, Nathoo, & Anisman, 2011) and rats (Toth, Avital, Leshem, Richter-Levin, & Braun, 2008). Together, these findings indicate that juvenile stress can have lasting effects on behavior in adulthood (Avital et al., 2006; Avital & Richter-Levin, 2005; Jacobson-Pick et al., 2011; Jacobson-Pick et al., 2008; Jacobson-Pick & Richter-Levin, 2010; Taylor et al., 2000; Toledo-Rodriguez & Sandi, 2007; Toth et al., 2008).

Consistent with the view that eating palatable foods may serve as a means of coping with distress in humans (Dube, LeBel, & Lu, 2005; Macht, 2008), in the present study palatable food mitigated the behavioral effects of the juvenile stress. In adulthood, chow-fed rats exposed to the stressor displayed significantly higher levels of anxiety-like behavior compared to their controls. This profile was not seen in stressed rats with access to the palatable diet. No significant differences occurred in closed arm entries across groups suggesting that the observed results were independent of locomotor activity. Similar results were obtained in the social interaction test with previously stressed chow-fed rats displaying reduced social behavior relative to their controls. Indeed, access to a palatable diet has been shown to mitigate the long term effects of maternal separation in the sucrose preference test and EPM (Maniam & Morris, 2010a), as well as the effects of short-term restraint stress in adulthood on anxiety-like behavior in the EPM (Pecoraro et al., 2004). Similar beneficial effects have been described among adult rats with

access to a sucrose solution following a restraint stress (Ulrich-Lai et al., 2010). Taken together with these earlier reports, our results provide evidence for the notion of palatable food consumption acts as a buffer against the negative behavioral effects of stress. Our results also provide preliminary evidence that such a diet may have proactive effects that are still evident in adulthood.

While no effects of the stressor were observed when animals were tested as juveniles, a diet effect in the EPM and social interaction test was observed, suggesting that the palatable diet may have had an anxiolytic effect. The lack of a stress effect among chow-fed rats contrasts with previous findings, as stressed juvenile rats were observed to display increased activity and exploration in both the open field and elevated plus maze which the authors characterized as "non-classic anxious behavior" (Jacobson-Pick and Richter-Levin, 2010, p. 274). One possible explanation for the observed differences between studies is the modification made to the stressor paradigm. Reducing the duration of restraint stress from 2 hours in the original protocol to 30 min may have diminished the acute effects of the stressor, resulting in a corresponding change in the behavior in the open field and EPM. Testing windows were also slightly different (24 hours post stress in the current study vs. 1-6 hours post stress).

Stressors ordinarily increase HPA activity (Greaves-Lord et al., 2007; Kallen et al., 2008) and palatable foods consumption has been associated with lower resting and stress-evoked cortisol levels in humans (Deuster, Singh, Hofmann, Moses, & Chrousos, 1992; Markus, Panhuysen, Tuiten, & Koppeschaar, 2000). In rodents, previous studies have reported normalized HPA activity in adrenalectomized (Bell et al., 2000; Bhatnagar et al., 2000; Laugero et al., 2001), reduced HPA axis response and hypothalamic corticotrophin releasing factor (CRF) mRNA expression following acute restraint stress (Foster et al., 2009) and diminished HPA axis

response to repeated restraint stress (Pecoraro et al., 2004) when animals are given access to a palatable diet. Moreover, a blunted corticosterone response, as well as reductions in hypothalamic CRF mRNA expression has been reported in response to restraint stress (Ulrich-Lai et al., 2010). HPA axis dampening is proposed to be a result of the hedonic properties of the sucrose rather than the increase caloric value, as reductions of plasma corticosterone in response to restraint are not observed when rats were given the same amount and schedule of sucrose solution via intragastric gavage (Ulrich-Lai et al., 2010). Consistent with previously reported results, we observed a significant decrease in basal plasma corticosterone concentrations among rats with access to palatable food.

Access to the palatable diet resulted in an overall increase in total caloric intake regardless of stressor exposure. Of greater interest, however, is the proportion of calories obtained from the palatable food to that of the standard chow. As stressor-induced preference for more pleasurable or palatable foods has been documented in both animals and humans (Dallman, 2010; Dallman et al., 2005; Pecoraro et al., 2004), we hypothesized that following the 3-day stressor, rats would show a preference for the palatable food. However, despite the mitigating effects of the palatable food on behavioral and neuroendocrine indices of anxiety, no such preference was observed. The lack of preference may be a result of the limited access to the palatable diet, as well as its availability during the light phase. Furthermore, as palatable food was provided in the morning prior to each stressor, it is possible that the timing of access to the palatable food might have influenced our results, as previous research has shown an increase in consumption when the palatable food is provided after the stressor (Foster et al., 2009). In addition, palatable preference in the present study was determined on the basis of intake per cage, rather than individual intake as rats were doubly housed. Although this was done to reduce stress stemming from isolation

(Einon & Morgan, 1977; Fone & Dixon, 1991; Weiss et al., 2004), the result of this decision is a reduction in statistical power, as well as confounding of individual eating patterns. Indeed, previous animal studies documenting a stress-induced increase in palatable feeding provided rats with *ad libitum* access to the favored diet (Dallman, 2010; Dallman et al., 2005; Pecoraro et al., 2004).

As expected, juvenile stressor exposure resulted in a decreased rate of weight gain, which is a well-established consequence of stressor exposure in rats (Marti et al., 1996). While initially both stress groups displayed a reduction in weight gain, rats with access to the palatable diet displayed a rate of weight gain comparable to their control group from PD 60 onwards, suggesting that access to the palatable food had a mitigating effect on this outcome. This pattern of recovered weight gain has been previously reported following restraint stress (Pecoraro et al., 2004).

Consistent with findings concerning the negative health consequences of high fat/sugar diets, rats with access to palatable food displayed increased adiposity compared to chow fed rats, despite the absence of any differences in body weight. Interestingly, juvenile stress was associated with a decrease in mesenteric fat pad weight among chow fed-rats only, suggesting that stress did not impact this fat depot among rats with access to the palatable diet. This may provide some support regarding how stress alters how fat is distributed throughout the body. It seems that when poor diet is coupled with chronic stress, reorganization of energy stores from peripheral storage to central storage, primarily as abdominal fat, is facilitated, accompanied by elevated levels of glucocorticoids and insulin (Dallman et al., 2005). High levels of abdominal fat have been associated with hypertension, cardiovascular disease, metabolic syndromes, type 2 diabetes, and morbidity and mortality (Friedman, 2003; Stunkard, Faith, & Allison, 2003).

However, selectively increased adiposity in the abdominal region is not reported in all studies that examined the effects of palatable food in the rat. Studies investigating the short-term effects of restraint stress (Foster et al., 2009; Pecoraro et al., 2004) and maternal separation (Maniam & Morris, 2010a) demonstrated general increases in fat pad weights among rats with access to palatable foods relative to chow fed rats, but not a selective increases in abdominal fat. Our results may be more germane to the long-term effects of access to a palatable diet. In the present study, rats were sacrificed six weeks following termination of the stressor and thus the observed differences in fat distribution between studies might be attributable to the prolonged access to the palatable diet.

High fat diets have been proposed as one of the factors which may lead to reduced insulin sensitivity, followed by insulin resistance and ultimately the development of type 2 diabetes (Spriijt-Metz, 2011). This has a corresponding effect on the ability of the body to process glucose. Reduced ability to tolerate a glucose load was not observed in the present study; however, the development of glucose intolerance following access to a high fat diet has been previously reported (Akerfeldt & Laybutt, 2011; Akyol, McMullen, & Langley-Evans, 2011; Cerf, 2007; Garg, Thakur, Alex, & Adamo, 2011). Exposure to a stressor may also contribute to the development of glucose intolerance. Dysregulation of the HPA axis as a result of stressor exposure, in conjunction with chronically elevated insulin levels, contribute to the development of insulin resistance, abdominal obesity, as well as metabolic syndrome (Pervanidou & Chrousos, 2011).

Conclusion

The present study provides additional evidence that stressor exposure during juvenility can have long lasting effects on anxiety-like behaviors in adulthood, and that access to palatable food

may have mitigating effects on the anxiety and corticosterone effects of juvenile stress. Not only do these results provide further support for the notion that palatable foods may be protective against the negative effects of stress, but also that these effects last into adulthood. However, the use of palatable foods as coping-strategy has long term negative effects on adiposity.

Disclosure

All of the authors declare that they have no direct or indirect conflicts of interests of a financial nature relevant to the present work.

Acknowledgements

This project was funded by an operating grant from the Canadian Institute for Health Research (CIHR) awarded to Zul Merali, and by a CIHR doctoral Canada Graduate Scholarship awarded to J. Christine MacKay. Technical contributions of William McLeod are gratefully acknowledged.

Preface to Chapter 5:

What is the long term impact of non-social episodic stressor exposure during adolescence when combined with social isolation via single housing conditions? Does access to a palatable high-fat, high-sugar diet mitigate the consequences of juvenile stress exposure among singly housed rats?

We have previously demonstrated that access to a palatable diet can mitigate the long-term consequences of exposure to a 3-day non-social stressor paradigm in juvenility (Mackay et al., 2014). In this study, previous exposure to juvenile stress was associated with anxiogenic profile in the elevated plus maze and social interaction test when rats were tested in adulthood. Access to a palatable diet was shown to mitigate these effects, as well as to dampen the effects of these stressors on basal HPA-axis functioning. However, consumption of the palatable diet led to negative long-term health consequences whereby a re-distribution of fat depots was observed among stressed rats with access to the palatable diet. In this regard, these rats displayed an increase weight of retroperitoneal fat pads, which was associated with a host of negative health consequences such as hypertension, cardiovascular disease, metabolic syndromes, type 2 diabetes, and morbidity and mortality (Friedman, 2003; Stunkard et al., 2003).

There is ample evidence in the literature to suggest that when given access to a high palatable high-fat, high-sugar food, exposure to stressor will encourage both rodents and humans to choose the more palatable choice (Dallman, 2010; Gibson, 2006; O'Connor et al., 2008; Zellner et al., 2006). This shift in preference has been proposed to serve as a form of coping or self-medication against the negative impacts of the stressor (Dallman et al., 2005). Interestingly, while previous work provided support for the stress dampening effects of access to a palatable diet, we failed to see a shift in preference towards the palatable diet (Mackay et al., 2014). This

was in part complicated by group-housing conditions which both reduced the sensitivity of our food measurements as well as reduced the sample sizes for our statistical analysis of feeding data. As such the present experiment sought to further characterize the potential stress-buffering effects of a palatable diet, as well as gain more insight into how stress may impact ingestive behaviors among singly-house animals.

Several individuals were involved in the design and implementation of this experiment, as well as the preparation and editing of the manuscript. Ms. Samantha Graitson assisted with the implementation of the experiment and made substantial contributes to the introduction of the present manuscript. Mr. Jonathan James assisted with data collection and the processing of endocrine and metabolic markers. Mr. Christian Cayer assisted with the analysis of data collected from behavioral assays. Dr. Pamela Kent assisted with data analysis and manuscript edits. Dr. Zul Merali provided guidance and input during the conceptualization and design of the study, as well as on-going feedback throughout data collection, analysis and manuscript preparation. Conceptual input regarding the results of the present experiment was provided by all authors. The author of this dissertation is the first author of the present manuscript and was involved in all aspects of the design and implementation of the experiments, as well as analysis of results and preparation of the manuscript.

Chapter 5: Exposure to sub chronic juvenile stress amid longstanding social isolation results in long-term behavioral consequences that are not mitigated by access to a palatable diet.

Authors

J. Christine MacKay

Samantha Graitson

Jonathan S. James

Christian Cayer

Pamela Kent

Zul Merali

Abstract

Approximately 15-20% of juveniles will encounter some form of trauma, with an increased risk of developing psychopathology. Stress has also been shown to promote palatable feeding. We have previously shown that access to a palatable diet can have mitigating effects on the long-term anxiety elicited by juvenile stressor exposure in group-housed rats. The current study sought to further characterize the long-term impact of juvenile stressor exposure and how access to a palatable die may mitigate these effects amongst animals housed individually. All rats had ad libitum access to chow or chow with 2 hr access to a palatable food. Male Wistar rats were exposed to 3 consecutive days of episodic stress on PD 27-29, while control rats received daily handling. Rats were then tested in elevated plus maze, social interaction, and forced swim test between PD 60-67. Results showed that exposure to juvenile stress results in a protracted behavioral pattern suggestive of depressive and anxiety symptomatology. However, this behavioral profile was not mitigated by palatable diet as we have previously demonstrated in group housed rats. These results suggest that stress dampening effect of a palatable diet may have an effectiveness "threshold" which the compounding effects of chronic social isolation and acute juvenile physical stress appear to have exceeded.

Keywords: Juvenile stress, Anxiety, Obesity, High fat diet, Social Isolation

Introduction

Obesity continues to be a growing health concern. Recent global estimates suggest that approximately 500 million adults are obese (International Obesity Task Force, 2010) and these numbers are projected to rise above 1 billion by 2030 (Kelly, Yang, Chen, Reynolds, & He, 2008). Obesity has been shown to contribute to and/or exacerbate outcomes of various health conditions including cardiovascular disease, type II diabetes, sleep apnea, as well as many psycho-social disorders (Knight, 2011; Stein & Colditz, 2004). At present obesity rates are higher in adults than children; however the rates of obesity among children and teens have almost tripled in the last 25 years (Gordon-Larsen, Adair, Nelson, & Popkin, 2004; Skelton, Cook, Auinger, Klein, & Barlow, 2009).

It is thought that the easy availability of highly palatable, calorie dense foods amid escalating levels of stress contributes to the frequency of obesity (Coccurello et al., 2009; Dallman, 2010; Dallman et al., 2005; Torres & Nowson, 2007). In this context, increased stressor experience and/or perceived stress may influence eating behavior and choices (amount and types of foods consumed) (Torres & Nowson, 2007; Wardle, Steptoe, Oliver, & Lipsey, 2000). Importantly, both humans and rodents increase their consumption of palatable food when stressed and the term 'comfort food' is often used to signify the stress-dampening properties of certain foods (Dallman, 2010; Dallman et al., 2005; Pecoraro et al., 2004). Children and/or adolescents may, in fact, be more amenable to resorting to coping with stressors by 'comfort eating', as other more cognitively based stress-coping mechanisms are not yet fully developed (Shimai, Kawabata, Nishioka, & Haruki, 2000; Sigman, 2003). Indeed, consumption of comfort foods is linked to improved emotional states (Dube et al., 2005) and lower resting and stress-

evoked cortisol levels (Deuster et al., 1992; Markus et al., 2000). These effects in humans are reproducible in animal models (Pecoraro et al., 2004; Ulrich-Lai et al., 2010).

Individuals may be particularly vulnerable to stress during certain developmental windows. For example, prenatal or neonatal stressor exposure can have profound and enduring effects on behavior and neurochemical functioning.(Graham et al., 1999; Weinstock, 1997) Evidently, early life events can alter the developmental trajectory of stress relevant mechanisms (e.g. through epigenetic processes) (Weaver et al., 2007). Most studies exploring the impact of early life stressors have focused on the early postnatal period (postnatal days (PD) 1-10). However, we and others have observed that the juvenile (early adolescent) period spanning PD 27-35 is exquisitely sensitive to stressors and has protracted behavioral and neurochemical ramifications that extend into adulthood (Avital & Richter-Levin, 2005; Jacobson-Pick et al., 2011; Jacobson-Pick et al., 2008; LeDoux, 1995).

We have recently found that exposure to stressors during the juvenile period is not only associate with long-term anxiety, but that this effect can be attenuated by consumption of a palatable diet (Mackay et al., 2014). Moreover, as in humans, resting levels of corticosterone were reduced in rats with access to the palatable or comfort foods, compared to those with access only to 'mundane' rat Chow. In addition to these stress buffering properties, palatable food consumption increased the accumulation of visceral fat among juvenile-stressed rats, indicating that the combination of stressor exposure during the juvenile period and access to palatable food may predispose individuals to developing obesity later in life. However, rats in this study were doubly housed making it challenging to evaluate feeding behaviors. Thus the purpose of the present study was to further characterize the long-term impact of juvenile stressor exposure and how access to a palatable diet may mitigate these effects amongst singly-housed animals. A

secondary objective was to gain a more accurate picture of feeding behavior following exposure to a sub chronic juvenile stress.

Materials and Methods

Animals. Forty-eight 21 day old male Wistar rats obtained from Charles River (Quebec, Canada). Rats were singly housed in standard plastic cages with bedding at a room temperature of 22±1°C on a 12 hour light-dark cycle. Weight gain was measured on PDs 21, 30, 40, 50, 60, and 70. All procedures met the guidelines established by the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Ottawa Institute of Mental Health Research.

Experimental design. An overview of the stress procedure and behavioural testing schedule is presented in Figure 15. Upon arrival, rats were randomly assigned to one of four conditions: (1) Chow + No Stress; (2) Chow + Stress; (3) Palatable + No Stress; and (4) Palatable + Stress. All animals underwent all behavioral tests, the air puff stressor challenge and glucose tolerance testing.

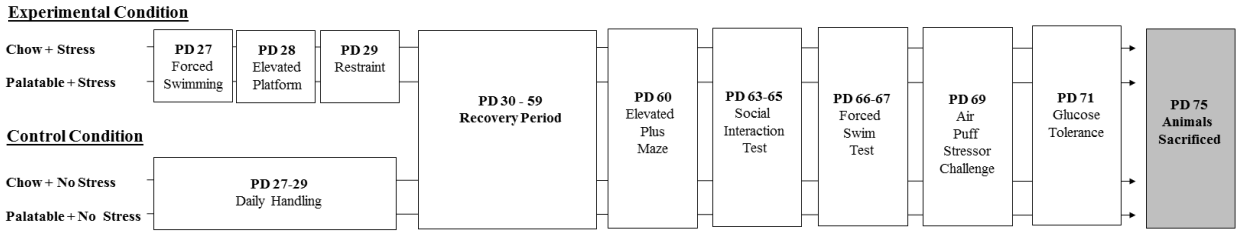

Figure 15. Summary of study design. Animals assigned to stress condition were exposed to a different stressor per day from PD 27-29 while control animals received daily handling. Animals were tested in adulthood following a 30-day recovery period.

Diet. All rats were given *ad libitum* access to standard laboratory chow (3.4 kcal/g, 4.5% fat, 18.1% protein, 57.3% carbohydrate, Charles River Rodent Diet 5075, Agribrand Purina Canada, Woodstock, Ontario, Canada) and rats assigned to the palatable food condition were given limited daily access to a 45%Kcal Fat diet (4.7 Kcal/g, 23.2% fat, 17.3% protein, 47.6% carbohydrates, TD.08811, Harlan Laboratories, Madison, Wisconsin, USA). Palatable food was given daily at 8:00 and removed at 10:00 and intake was measured. On PDs 21, 30, 40, 50, and 60, Chow consumption was measured by subtracting the weight of the chow in the cage from its weight 24-hours earlier. Total caloric intake was calculated by multiplying the energy content (Kcal/g) of individual diets by the amount consumed and summing them. Calories from the palatable diet were calculated by multiplying the amount of food consumed by the energy content of the diet. Food intake data was collected from PD 21 to PD60.

Juvenile stress paradigm. The juvenile stress procedure used was a modification of the 3 day procedure described by Jacobson-Pick and Ritcher-Levin (2010). This procedure comprised 3 consecutive days of exposure to a different stressor per day throughout PD27-29.

PD 27 - Forced swimming. Rats were individually placed in a circular water basin (diameter 48cm; height 42cm; water depth 29 cm and a temperature of 22±2°C) for 10 min, during which they swam or floated continuously.

PD 28 – Elevated platform. Rats were individually placed on a small elevated platform (12cm x 12cm; 70cm elevation from water level) for three separate 30 min sessions. Platforms stood within a basin filled with water for the animals' protection should they fall off. During the intersession interval of 90 min rats were returned to their home cage. Animals that fell during the testing session were immediately returned to the platform for the remainder of the test session.

PD 29 – Restraint. Rats were placed for 30 min in a plastic restraining bag that prevented side-to-side movement and limited forward-backward movement. The plastic bag had a hole at the end closest to the rat's nose to allow for ventilation.

Behavioral testing. Behavioral testing was conducted under low illumination (30 - 40 lux) between 10:00-13:00 daily following a 1 hr period during which rats habituated to the testing room. Behavior was monitored via a video camera mounted above the arena.

Elevated plus maze. On PD 60, rats were placed on the center platform of the EPM (facing a closed arm), immediately after OF testing. The EPM consisted of two open arms (50 x 10 cm) and two perpendicularly situated arms enclosed by 40 cm high walls, elevated approximately 66 cm above the floor. Behaviors scored during the 5 min test included time spent on the open and closed arms, risk assessment behavior (unprotected head dips; head protruding over the edge of an open arm) and number of entries into the closed arm. Time in the open arms and unprotected head dips are validated measures of anxiety-like behavior (Carobrez & Bertoglio, 2005). The number of entries into the closed arms of the maze are viewed as a measure of spontaneous locomotor activity (Walf & Frye, 2007).

Social interaction. The social interaction test was conducted over a total duration of three days. On habituation day 1 (PD 63), rats were matched to a partner from the same Diet x Stress condition (but from a different cage) on the basis of body weight, such that members of a pair did not differ by >10g. Paired rats were placed in the arena (60 x 60 cm; 30cm high walls) together for five minutes. On habituation day 2 (PD 64), all rats were individually placed in the arena for a period of three min. On test day (PD 65), the same pairs of rats used on habituation day 1 were placed in the arena and social behaviors of both rats were observed for 7 min. Total time spent in social interaction (including sniffing, climbing over each other, following,

allogrooming, and play fighting) was recorded and each individual analyzed separately. Decreases in social interaction in rodents may be reflective of an anxiogenic profile (File & Seth, 2003).

Forced swim test. The forced swim test is a widely used behavioral despair paradigm used to evaluate the effectiveness of antidepressant drugs (Porsolt et al., 1978; Porsolt et al., 1977; Schiller et al., 1992). The forced swim arena consisted of a clear Plexiglas cylinder (18 x 60 cm, water height 35 cm, temperature 25 °C; Stoelting Co., Wood Dale, Illinois). A habituation session (PD 66: 15 min) was performed twenty-four hours prior to the test session. On test day (PD 67) the animal was returned to the same tube for 5 minutes. The time spent immobile, was recorded during the test session. Immobility was defined as an animal floating in the water without struggling, and only engaging in movements to keep the head above water (Slattery & Cryan, 2012). Increases in immobility are thought to reflect behavioral despair (Cryan et al., 2002; Lucki, 1997). Therefore reduced immobility is used to assess the antidepressant properties of a given compound (Cryan et al., 2005).

Corticosterone response to a novel stressor. On PD 69, all rats were exposed to a novel stressor via the application of air-puff stress. This stress consisted of a 5 sec puff of air from an aerosol can into the rat's face, delivered once a minute for 5 minutes. Blood samples, collected using tail venipuncture, were taken immediately before the stressor, and 20, 30, 45 and 75 min post-stressor exposure. Blood droplets were deposited onto 903 Protein Saver filter paper (GE Healthcare Bio-Sciences Corp, MA, USA), allowed to dry at room temperature then stored at -20°C.

Collected samples were analysed using a commercial radioimmunoassay (RIA). Two days prior to the RIA procedure, blood was eluted from the filter paper by placing one 3 mm punch

(per time point) of filter paper in a 12 x 75 culture tube containing 200 µL Dulbecco's Phosphate Buffered Saline (Sigma, item D-5773) w/0.1% gelatine, covered with parafilm in a fridge at 4°C. On the day of the RIA procedure, culture tubes containing the samples were placed on an orbital shaker for 1 hour at room temp. Corticosterone levels were the determined from the eluted blood sample using commercial RIA kits as per the manufacturer's instructions (MP Biomedicals, CA). The inter- and intra-assay variability was 11.02% and 9.96%, respectively.

Glucose tolerance. On PD 71, following 12hr of fasting, all rats were given an intraperitoneal injection (IP) of a 0.75g/mL dextrose solution (dose: 1.75g/kg). Blood glucose was measured by applying a drop of blood (via tail venipuncture) onto a test strip, then taking a reading with a blood glucose meter (Accu-Chek Aviva Nano, Roche Diagnostics, Mannheim, Germany). Levels were assessed immediately before the injection, and 15, 30, 60 and 120 min post-injection.

Adiposity. Following sacrifice on PD 75, animal carcasses were stored at -20°C. Frozen carcasses were thawed to room temperature and weighed. Quantitative magnetic resonance imaging (EchoMRI, Echo Medical Systems, Houston, TX) was used to determine the body composition (fat, lean mass, and water) of the carcasses.

Statistical analysis. Data obtained from the EPM, social interaction, FST, and adiposity were analyzed by 2-way analysis of variance (ANOVA) where Diet (2 levels; chow vs. palatable food) and Stress (2 levels; stress vs no stress) were the between group factors. Body weight was analyzed using 3-way repeated-measures ANOVAs, where Diet (2 levels) and Stress (2 levels) were the between group variables and Time (6 points) the repeated within group variable. Total caloric intake was analyzed using 3-way repeated-measures ANOVAs, where Diet (2 levels) and Stress (2 levels) were the between group variables and Time (10 points) the repeated within

group variable. Calories from the palatable diet was analyzed using 2-way repeated-measures ANOVAs, where Stress (2 levels) was the between group variable and Time (10 points) the repeated within group variable. Corticosterone response to a novel stressor and glucose tolerance results were analyzed using 3-way repeated-measures ANOVAs, where Diet (2 levels) and Stress (2 levels) were the between group variables and Time (5 points) the repeated within group variable.

Subsequent follow-up comparisons were conducted using *t* tests with a Bonferroni correction to maintain the alpha level at .05. For some measures a priori predictions that access to the palatable diet would alter the impact of the stressor were made. Analyses of the simple effects for interactions and a priori predictions were conducted irrespective of whether the F value for an interaction reached significance (Winer, 1962), especially when visual inspection of graphed results suggested that a significant difference may exist. Data points ±3 standard deviations from calculated means were considered as outliers and not included in statistical analysis (Taylor, 1997). Some rats were removed from the statistical analysis of variable due to missing data, thus the N and df associated with these measures vary across outcomes.

Results

Effect of stress and diet on body weight and food intake. Repeated measures ANOVA revealed a significant Diet x Time interaction for weight gain, $F_{Diet\ x\ Time}(5, 215) = 8.713$, $p < .01$; Figure 16A. No significant effect of Stress x Time, $F_{Stress\ x\ Time}(5, 215) = .092$, $p = .993$, and interaction between Stress x Diet x Time was observed, $F_{Diet\ x\ Stress\ x\ Time}(5, 215) = .429$, $p = .828$. As expected, simple effect revealed that all rats gained weight over time. In addition, those with access to the palatable diet, in general, weighed more than Chow fed rats; a difference that reached significance by PD 70. Repeated measures ANOVA revealed no significant effect of

Stress x Time, $F_{Stress\ x\ Time}$ (9,396) = 1.38, p = .195, and Diet x Time, $F_{Diet\ x\ Time}$ (9,396) = .819, p = .598 on total caloric intake (Figure 16B). The interaction between Stress x Diet x Time on total caloric intake was also non-significant, $F_{Diet\ x\ Stress\ x\ Time}$ (9,396) = .886, p = .538. No significant effect of Stress was observed in calories from the palatable food (Figure 16C), $F_{Stress\ x\ Time}$(9,396) = 1.011, p = .432.

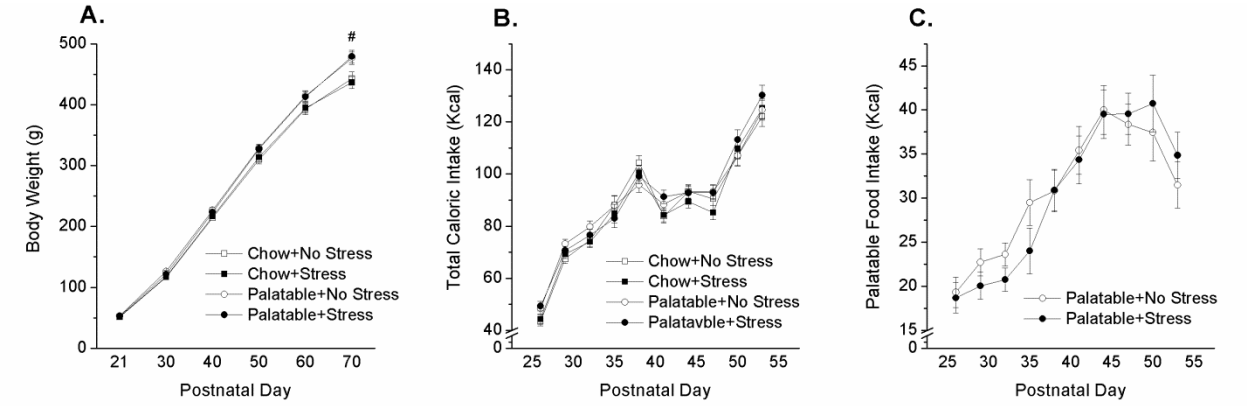

Figure 16. Effect of juvenile stress and diet on body weight and feeding behavior. While there were no differences in calorie intake, rats with access to the palatable diet weighed more than their Chow fed counterparts by PD 70. Data represents mean ± SEM. # Significant effect of Diet at $p < .05$;

Effect of stress and diet on anxiety- and depressive-like behavior. Juvenile stress induced long-term anxiety-like behavior on the elevated plus maze (Figure 17A-D). Two-way ANOVA indicated a significant main effect of Stress, F_{Stress}(1,43) = 4.547, p = .039, and no effect of Diet, F_{Diet} (1,43) = .381, p = .540, on time spent in the closed arm; the interaction between Stress and Diet was non-significant $F_{Stress\ x\ Diet}$ (1,43) = 1.16, p = .288. Similarly, a significant effect of stress was observed in time spent in the open arm of the maze F_{Stress}(1,43) =

10.353, $p = .002$. The main effect of Diet, $F_{Diet}(1,43) = .009$, $p = .923$ and interaction between Stress x Diet, $F_{Stress\ x\ Diet}(1,43) = .021$, $p = .886$ were also non-significant for time spent in the open arm. A significant effect of Stress was also observed in the number of risk assessments, $F_{Stress}(1,43) = 4.798$, $p = .034$. The main effect of Diet, $F_{Diet}(1,43) = .015$, $p = .904$ and interaction between Stress x Diet, $F_{Stress\ x\ Diet}(1,43) = .003$, $p = .955$ were non-significant. Follow-up simple effects analyses were completed based on the a priori hypothesis that at significant Stress x Diet interaction would be present for these variables. Comparisons indicated that all stressed rats spent significantly less time on the open arms of the maze regardless of diet. As see in Figure 19A, the significant effect of Stress was only observed in Chow-fed rats. No main effect of Stress, $F_{Stress}\ (1,43) = .342$, $p = .562$, and Diet, $F_{Diet}\ (1,43) = .014$, $p = .905$, were observed in the number of entries into the closed arm was observed across groups. The interaction between Stress and Diet was also non-significant, $F_{Stress\ x\ Diet}\ (1,43) = .720$, $p = .40$, suggesting no differences in locomotor behaviour across groups.

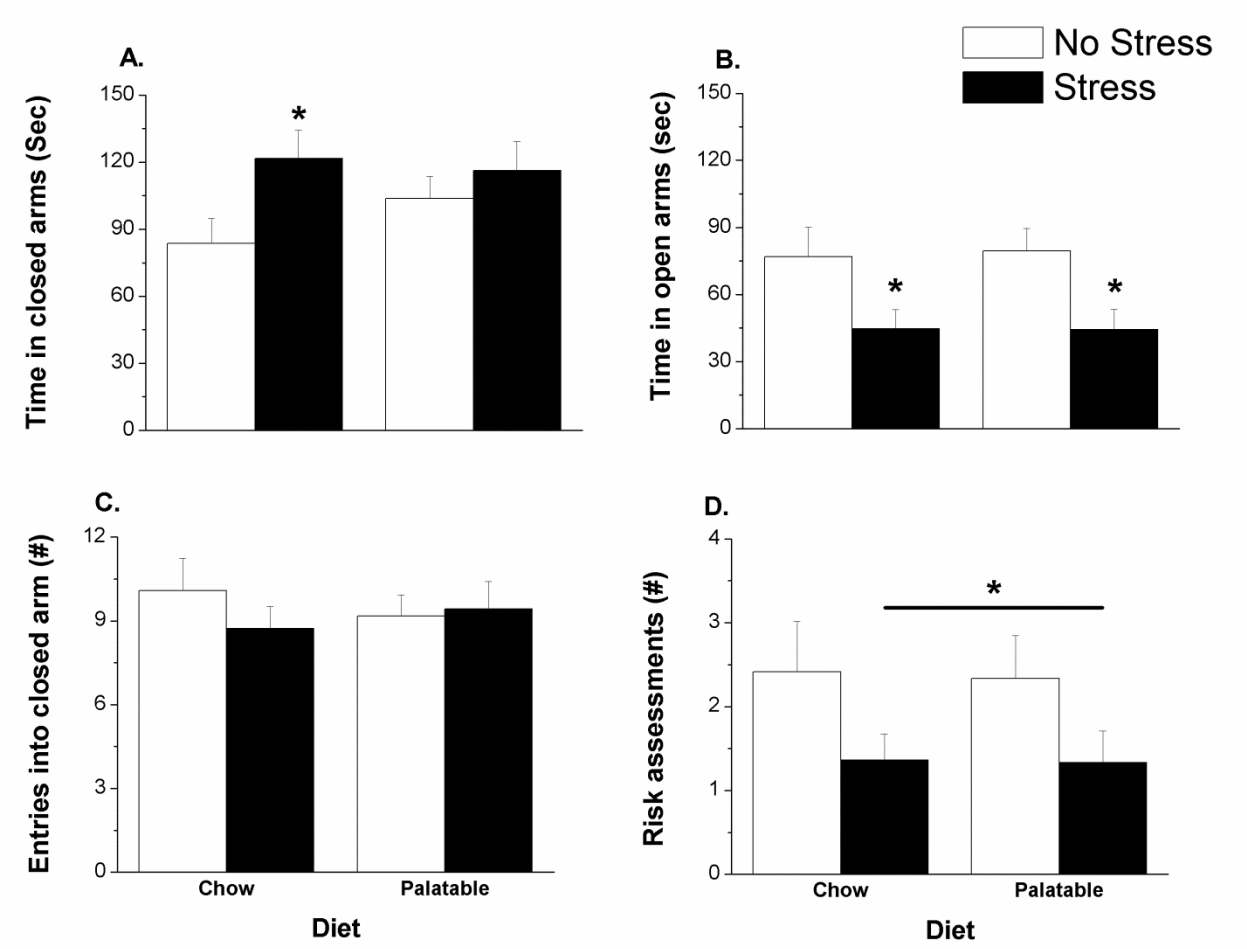

Figure 17. Effect of juvenile stress and diet on behavior in the elevated plus maze. All stressed rats spent significantly less time in the open arms of the maze and made fewer risk assessments regardless of diet. Bars represent mean ± SEM * Significant effect of Stress at $p < .05$.

Figure 18 depicts the total time spent engage in active social interaction (A) and a breakdown of the individual behaviors encompassed within the total (B). Previous exposure to juvenile stress was associated with a decrease in social behaviors in adulthood and two-way ANOVA revealed a significant Stress effect for overall time spent in social interaction, $F_{Stress}(1,44) = 15.16$, $p < .001$; Figure 18A. No main effect of Diet was observed, $F_{Stress}(1,44) =$

2.52, $p = .119$, and the interaction between Stress and Diet was non-significant, $F_{Stress\ x\ Diet}(1,44) = 035$, $p = .853$.

ANOVAs for the individual behaviors also revealed significant Stress effects for time spent sniffing $F_{Stress}(1,44) = 10.79$, $p = .002$; Figure 18B; moving over/under the partner $F_{Stress}(1,44) = 9.79$, $p = .003$; Figure 18B; and play fighting $F_{Stress}(1,44) = 6.02$, $p = .018$; Figure 18C, as these behaviors were decreased in previously stressed rats irrespective of diet. The main effect of Diet, and the interaction between Stress and Diet were both non-significant for time spent sniffing [$F_{Diet}(1,44) = .685$, $p = .412$ and $F_{Stress\ x\ Diet}(1,44) = .212$, $p = .647$]; moving over/under the partner [$F_{Diet}(1,44) = .469$, $p = .497$ and $F_{Stress\ x\ Diet}(1,44) = .056$, $p = .814$]; and play fighting [$F_{Diet}(1,44) = 1.05$, $p = .310$ and $F_{Stress\ x\ Diet}(1,44) = 1.51$, $p = .226$]. A significant Diet, $F_{Diet}(1,44) = 7.50$, $p = .009$, and Stress, $F_{Stress}(1,44) = 8.56$, $p = .005$, effect was observed for time spent following the partner (Figure 18C) as baseline levels of following were greater in palatable food fed rats, yet previous stress exposure decreased this behavior in both diet conditions. The interaction between Stress and Diet for following was non-significant, $F_{Stress\ x\ Diet}(1,44) = .260$, $p = .613$. No differences in allogrooming were observed, $F_{Stress}(1,44) = .598$, $p = .443$, $F_{Diet}(1,44) = .078$, $p = .782$, and $F_{Stress\ x\ Diet}(1,44) = .436$, $p = .51$.

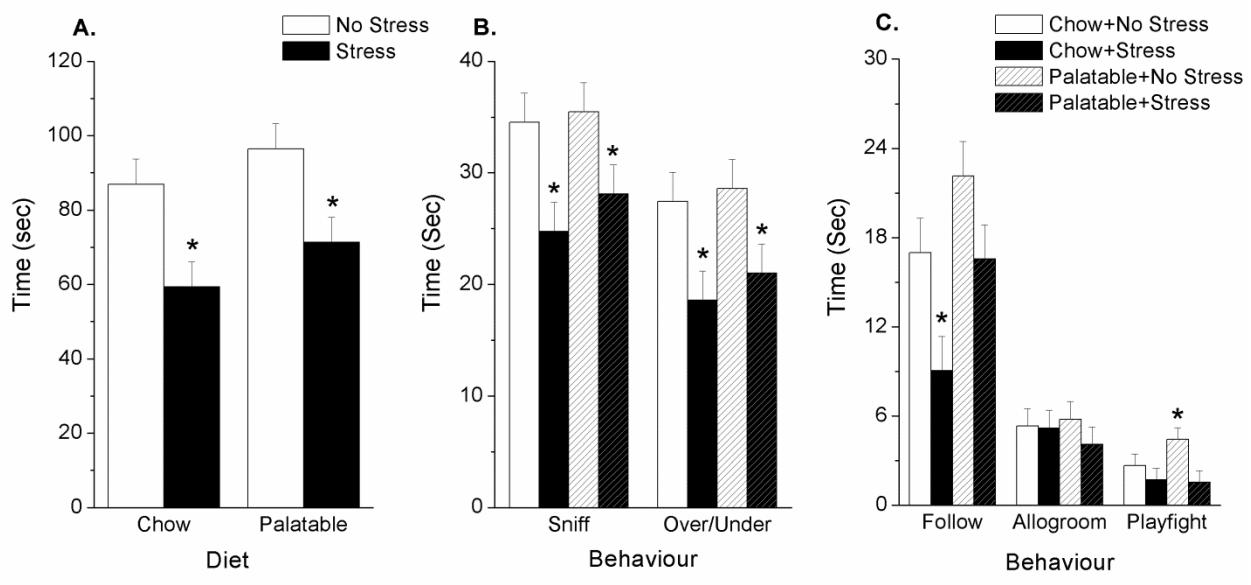

Figure 18. Effect of juvenile stress and diet on behavior in the social interaction test. Previous exposure to stress in juvenility was associated with a decrease in total time spent engaging in social behaviors, as well as time spent sniffing and moving over/under their partner. Bars represent mean ± SEM * Significant effect of Stress at $p < .05$.

In general, previously stressed rats display reduced activity in the FST (Figure 19). Two-way ANOVA revealed by a significant effect of Stress, $F_{Stress}(1,43) = 19.04$, $p < .001$,and no effect of Diet, $F_{Diet}(1,43) = 1.35$, $p = .252$, on total time spent immobile. The interaction between Stress and Diet was non-significant, $F_{Stress\ x\ Diet}(1,43) = 1.63$, $p = .208$.

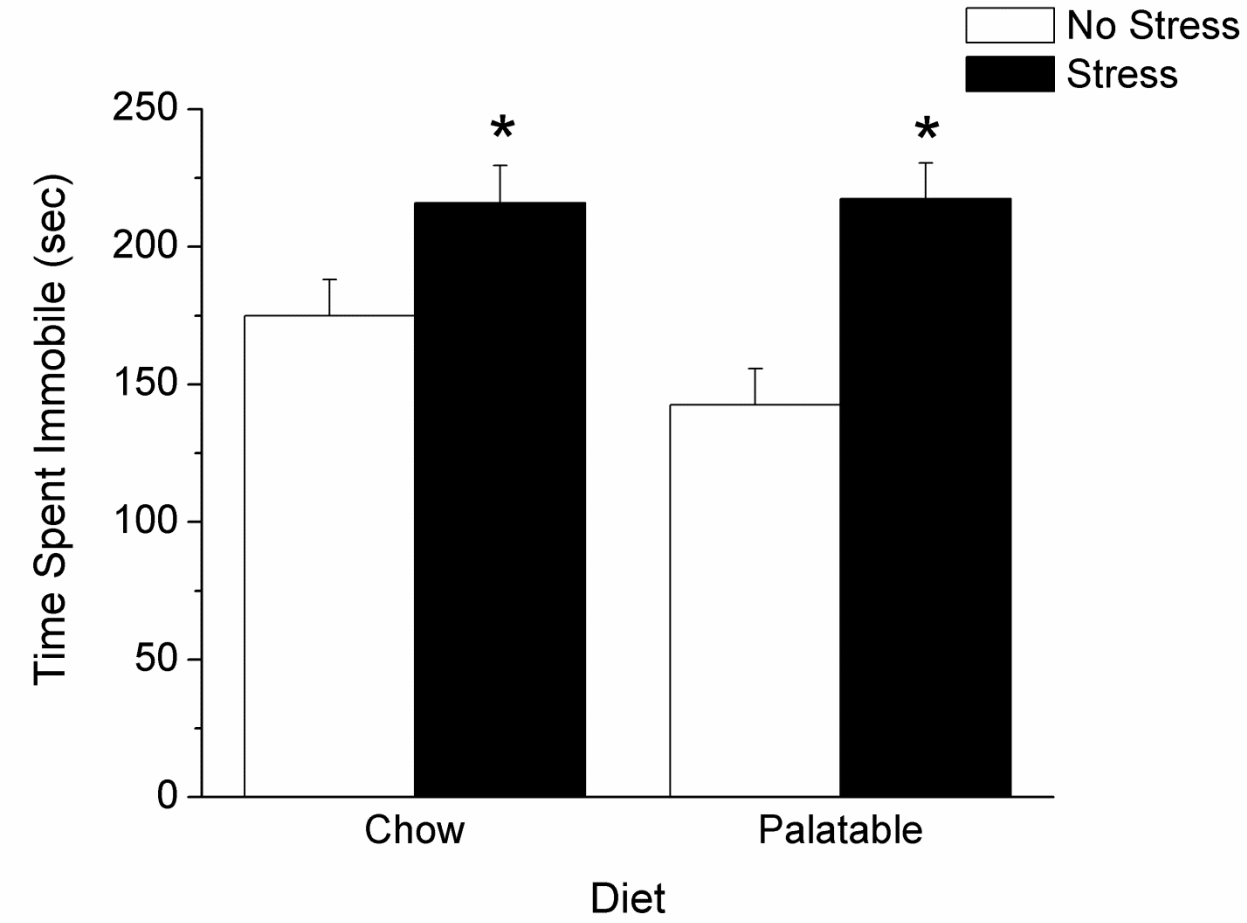

Figure 19. Impact of juvenile stress and diet on behavior in the forced swim tests. Exposure to juvenile stress was associated with increased immobility in the FST. Bars represent mean ± SEM

* Significant effect of Stress at $p < .05$

Effect of stress and diet on endocrine and metabolic indices. Repeated measures ANOVA revealed no significant effect of Diet x Time, $F_{Diet\ x\ Time}(4,168) = .701$, $p = .592$, and Stress x Time, $F_{Stress\ x\ Time}(4,168) = .849$, $p = .496$, on corticosterone response to an air puff stressor. Similarly, the interaction between Stress x Diet x Time was non-significant, $F_{Stress\ x\ Diet\ x\ Time}(4,168) = .826$, $p = .510$; Figure 20A. However, post hoc analysis was completed based on an

a priori assumption that a significant Stress x Diet x Time interaction would be present and on visual inspection of graphed results. Follow-up comparisons indicated that prior to the air puff stress previously stressed rats with access to chow only displayed increased basal corticosterone relative to their controls. A significant elevated in corticosterone was also observed 25min after the air puff stress.

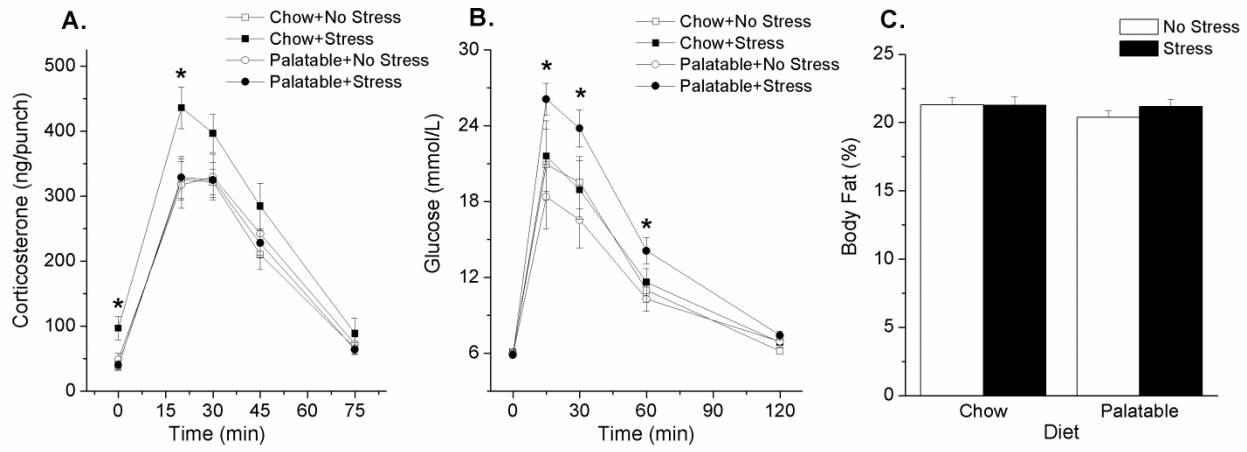

Figure 20. Impact of stress and diet on endocrine and metabolic indices. Stressed rats with access to chow displayed in increase in basal corticosterone, as well as an elevated corticosterone response 15 min after stressor presentation (A). Access to the palatable and previous stressor exposure was associated with glucose intolerance (B). No differences were observed in percentage of body fat (C). Data represents mean ± SEM * Significant effect of Stress at $p < .05$.

A significant Diet x Stress x Time interaction was observed in the rats ability to tolerate a glucose load, $F_{Diet\ x\ Stress\ x\ Time}$ (4,172) = 2.641, p = .035; Figure 20B. Simple effects analysis indicated that relative to their controls, previously stressed rats with access to the palatable diet

displayed significantly elevated blood glucose at 15, 30 and 60 min following an IP injection of a dextrose solution.

Two-way ANOVA indicated no significant main effect of Stress, F_{Stress} (1,43) = .683, p = .413, and Diet, F_{Diet} (1,43) = .608, p = .440, on total body fat, and the interaction between Stress and Diet was non-significant $F_{Stress\ x\ Diet}$ (1,43) = .419, p = .035; Figure 20C.

Discussion

The aim of the present study was to further characterize the long-term impact of physical stress during juvenility among singly-housed rats and, in particular, whether or not access to a palatable diet may mitigate these effects. We have previously reported that access to a palatable diet can mitigate the long-term impact of juvenile stress in group housed animals as increased anxiety-like behavior was evident in rats that consumed chow only and not those with daily access to palatable food (Mackay et al., 2014). In the present experiment, although chow fed rats displayed increased anxiety-like behavior in adulthood as previously observed, access to the palatable diet did not attenuate this effect in singly-housed animals. Decreased time in the open arms EPM and decreased risk assessment were observed in the EPM among all stressed rats irrespective of diet, which was concurrent with decreased sociability in the social interaction test. Furthermore, in the FST, all rats previously exposed to juvenile stress displayed increased immobility behavior suggesting that the palatable diet in this study did not have an anti-depressant effect among stressed rats. As behavioral results among control rats within the present study were relatively similar, it is possible that single-housing conditions may have served as an additional long-term stressor on top of the acutely applied juvenile stress. Interestingly, access to a palatable diet was unable to mitigate the behavioral impact of isolation between PD 21 to PD 28 on the EPM and open field when animals were tested in adulthood (Arcego et al., 2013).

Similar results were observed with assessment of the impact of neo-natal handling and social isolation in juvenility, whereby social isolation led to an anxiogenic profile in the EPM regardless of diet (Marcolin et al., 2012). As such, the present findings may speak towards compounding impact of single-housing conditions.

Social isolation in juvenility has been associated with a variety of long-lasting neurochemical and behavioral alterations in isolates relative to group-housed counterparts (Einon & Morgan, 1977; Heidbreder et al., 2000; Lapiz et al., 2003; Valzelli, 1973). Interestingly, these alterations are only evident when rodents are isolated during a critical window (PD 20 to 30) close to pubertal onset (Einon & Morgan, 1977; Robbins et al., 1996), as observed results of isolation in young rats are not seen in adult rats when the same procedure is employed (Fone & Porkess, 2008). Social isolation during adolescence has been consistently linked with increases in anxiety-like behavior in the elevated plus maze. For example, Long Evans rats isolated from PD 28 spent less time in the open arms, and made less open arm entries in the EPM when tested between PD 72-78 (Chappell et al., 2013). Increase anxiety-like behavior in the EPM was evident 13 weeks after social isolation beginning at PD 21 (Weiss et al., 2004). Increased anxiety-like behavior in adulthood following isolation in juvenility has been reported by others (Hall, 1998; Hall et al., 1998; Hall et al., 2000; Hellemans et al., 2004; Jankowska et al., 1991; Lodge & Lawrence, 2003; Maisonnette et al., 1993; Parker & Morinan, 1986; Wright et al., 1991; Wright et al., 1991). The impact of isolation on social behavior appears to vary based on whether or not rats are re-socialized following isolation. Most germane to the present study are those that remain isolated. Studies involving chronic isolation with no re-socialization have reported that isolates display an increase in social interaction with novel conspecifics in adulthood (Ferdman et al., 2007; Ikemoto & Panksepp, 1992; Niesink & Van Ree, 1982a;

Niesink & Van Ree, 1982b; Rockman et al., 1987; Wongwitdecha & Marsden, 1996). This appears to be inconsistent with present results; however, these studies did not have the additional variable of an acute physical stressor. As with social interaction, depressive-like behaviors following adolescent social isolation are also inconsistent with some studies reporting impairments in the FST (Brenes et al., 2008), and other reporting no impact (Hall et al., 1998; Hall et al., 2001; Hong et al., 2012).

Decreased rate of weight gain is a well-established consequence of stressor exposure in rats (Marti et al., 1996). We have previously reported a decreased rate of weight gain amongst group-housed rats with access chow only following exposure to the same sub chronic juvenile stressor (Mackay et al., 2014). Access to a palatable diet in that study was shown to have a mitigating effect. Interestingly, the same juvenile stress exposure did not appear to impact body weight gain in the present study as singly-housed stressed rats displayed a similar rate of weight gain relative to their controls. The lack of observed stress effects on rate of weight gain in the present study suggests that housing conditions may have played a role. As such, the results of the present study may be impacted by the potential confounding effects of longstanding social isolation which could have obscured the effects of juvenile stress. While the large majority of studies investigating isolation housing from weaning report minimal changes in body weight relative to age-matched socially housed rats (Hellemans et al., 2004), rats with access to the palatable diet in the present study displayed an increased rate of weight gain irrespective of juvenile stressor exposure. This observed difference would seem to suggest that exposure to longstanding social isolation may have had some discernable impact on rate of weight gain, and one could hypothesize that access to the palatable diet served to mitigate this effect.

In terms of HPA axis response to a novel stressor, all rats displayed a robust increase in corticosterone following the application of a novel air-puff; however stressed rats with access to chow only displayed enhanced basal corticosterone levels prior to stressor exposure relative to both their controls and stressed rats with access to the palatable diet. This exaggerated HPA-axis activity carried forward following stressor exposure, where significantly elevated corticosterone levels were observed 30 minutes following the stressor exposure. As this pattern was only observed amongst stressed rats with access to chow only, it is possible that access to the palatable attenuated HPA-axis response to the stressor amongst the other stress group. Such findings would be consistent with other reports as access to a palatable diet has been associated with reduced hypothalamic CRF mRNA (Foster et al., 2009; Ulrich-Lai et al., 2010) and diminished HPA axis response to restraint stress (Pecoraro et al., 2004; Ulrich-Lai et al., 2010).

A secondary objective of the present study was to evaluate how exposure to a stressor may impact consumption of a palatable food. Indeed, stressor-induced preference for more pleasurable or palatable foods has been documented in both animals and humans (Dallman, 2010; Dallman et al., 2005; Pecoraro et al., 2004). Consistent with previously reported results in group-housed animals (Mackay et al., 2014), exposure to juvenile stress did not appear to influence palatable food intake. There also appeared to be no significant differences in total caloric intake across groups. The present results seem to be inconsistent with the concept of "self-medication" with food, as stressed rat did not increase their intake of the palatable diet; however, it is possible that this relates to methodological variation. In particular, the palatable diet in the present study was distributed in a pellet form whereas previous studies have provided individual allocations of each food component (e.g. fat, protein, carbohydrates) (Dallman, 2010; Dallman et al., 2005; Pecoraro et al., 2004), or even access to processed snack foods available to

humans (e.g. cakes, biscuits) (Maniam & Morris, 2010a). As such, the relative concentration of each food energy component appears to vary across studies. The lack of preference may also be a result of the limited access to the palatable diet, as well as its availability during the light phase and the timing of administration (e.g. before the stressor). Previous research has shown an increase in consumption when the palatable food is provided after the stressor (Foster et al., 2009).

Access to the palatable diet did not impact body fat percentage, which was somewhat unexpected and inconsistent with our previously reported results in group-housed animals as rats with access to the palatable displayed increased adiposity in the mesenteric, retroperitoneal and subcutaneous inguinal white fat pads (Mackay et al., 2014). Additionally, stressed rats with access to the palatable diet displayed increased mesenteric fat pad relative to those with access to chow only, suggesting that stress may have altered how fat was distributed through the body. While the present results suggest that there were no discernable differences in body fat, it is possible that the methods used to assess adiposity may not have afforded the necessary specificity. Indeed increased adiposity in retroperitoneal and gonadal fat pads has been reported among rats with access to a palatable diet and this is proposed to be further exacerbated by previous exposure to pre-pubertal social isolation (Arcego et al., 2014; Krolow et al., 2013a). While we did not find differences in adiposity, there is evidence to suggest that access to the palatable diet may have contribute to glucose intolerance amongst previously stressed rats. The development of glucose intolerance following access to a high fat diet alone has been previously reported (Akerfeldt & Laybutt, 2011; Akyol et al., 2011; Cerf, 2007; Garg et al., 2011).

In sum, behavioral results of the present study appear to be in contrast to what we have previously reported in group-housed animals exposed to the same sub-chronic stressor during

juvenility (Mackay et al., 2014). In particular, access to the palatable diet did not appear to mitigate the long-term behavioral consequences of juvenile stressor exposure when it is applied in conjunction with the longstanding social isolation brought about by single-housing conditions. It is possible that the stress dampening effect of the palatable diet may have an effectiveness "threshold" which the compounding effects of chronic social isolation and acute juvenile physical stress appear to have exceeded. Interestingly, there is some evidence to suggest that access to the diet may have mitigated some of the physiological effect of juvenile stress pertaining to HPA-axis functioning and reactivity. As such, there may be differing sensitivities of behavioral and physiological parameters to the comforting effects of a palatable diet.

Disclosure

All of the authors declare that they have no direct or indirect conflicts of interests of a financial nature relevant to the present work.

Acknowledgements

This project was funded by an operating grant from the Canadian Institute for Health Research (CIHR) awarded to Zul Merali, and by a CIHR doctoral Canada Graduate Scholarship awarded to J. Christine MacKay. Technical contributions of Laura Kenney at Health Canada and Krista Gruenwald are gratefully acknowledged.

Preface to Chapter 6

What is the long term impact of juvenile stress exposure (episodic stressor and social isolation) combined with palatable food diet on brain neurochemistry? Specifically, how does stress and diet impact the expression of dopamine receptors in the pre-frontal cortex and nucleus accumbens?

The decision to eat or not to eat involves several internal and external factors, including energy homeostasis, emotional factors (i.e. hedonic aspects of food) and environmental cues (i.e. food availability, stressor exposure) (Dallman, 2010; Levine & Billington, 1997; Pliner & Mann, 2004; Popkin, Duffey, & Gordon-Larsen, 2005). Most conceptualizations of feeding regulation propose that two parallel systems interact to mediate these complex interactions (Kelley, Baldo, Pratt, & Will, 2005; Lutter & Nestler, 2009). On one level, there is the homeostatic system encompassing hypothalamic and brainstem circuits that control energy balance. Based on nutrient availability and metabolic needs, peptidergic signals are utilized to initiate or inhibit feeding with the purpose of maintaining energy homeostasis. These classic feeding pathways are embedded in a much larger neural circuitry referred to as the cortico-limbic or non-homeostatic system.(Kelley et al., 2005; Lutter & Nestler, 2009) This system (encompassing the nucleus accumbens (NAc), anterior cingulated cortex (ACC) and amygdala) coordinates metabolic needs with external factors, including external challenges, memories, habits and pleasurable feelings.(Kampe, Tschop, Hollis, & Oldfield, 2009; Zheng, Lenard, Shin, & Berthoud, 2009) Factors beyond hunger/metabolic demand do promote feeding, as underscored by the fact that both humans and rodents will consume palatable foods well beyond the point when energy demands have been met. The powerful rewarding properties of food have been paralleled to drugs of abuse, causing some researchers to refer to overeating as an addiction,(Avena & Gold, 2011; Dagher, 2009) and

pointing to the importance of cortico-limbic circuitry (containing many key reward sites) in the control of food intake.

It has been suggested that the mechanism(s) providing stress relief by palatable food consumption likely resides at the intersection of metabolic, reward and stress regulatory circuitry (Ulrich-Lai et al., 2010). As discussed above, key nodes within these circuitries include hypothalamic sites (paraventricular (PVN) and arcuate nuclei) and cortico-limbic sites (the ACC, the basolateral and central amygdala and NAc). In order to begin to delineate the cellular mechanism(s) involved in the stress relief provided by palatable foods, the experiment in Chapter 6 sought to investigate how chronic palatable food intake affects stressor-induced changes in the mRNA expression of the dopamine receptors in the PFC and NAc.

Several individuals were involved in the design and implementation of this experiment, as well as the preparation and editing of the manuscript. Ms. Eliza Ali and Dr. Marie-Claude Audet made substantial contributions to quantitative polymerase chain reaction (qPCR) analyses including sample preparation and trouble-shooting. Additionally, Ms. Ali assisted with data collection and manuscript preparation. Dr. Audet also provided assistance with manuscript edits. Mr. Jonathan James assisted with data collection and the processing of endocrine and metabolic markers. Mr. Christian Cayer assisted with the analysis of data collected from behavioral assays. Dr. Pamela Kent assisted with data analysis and manuscript edits. Dr. Zul Merali provided guidance and input during the conceptualization and design of the study, as well as on-going feedback throughout data collection, analysis and manuscript preparation. Conceptual input regarding the results of the present experiment was provided by all authors. The author of this dissertation is the first author of the present manuscript and was involved in all aspects of the

design and implementation of the experiments, as well as analysis of results and preparation of the manuscript.

Chapter 6: Access to a palatable diet amid exposure to juvenile stress results in long-term changes in D_1 and D_2 receptor expression in the nucleus accumbens.

Authors

J. Christine MacKay

Eliza Ali

Marie-Claude Audet

Jonathan S. James

Christian Cayer

Pamela Kent

Zul Merali

Abstract

We have previously shown that access to a palatable diet can have mitigating effects on the long-term anxiety elicited by juvenile stressor exposure in group-housed rats. It has been suggested that the mechanism(s) providing stress relief by palatable food consumption may relate to its rewarding properties The present study sought to characterize long-term impacts of juvenile stress on DA receptor expression in the NAc and PFC and the potential stress-reducing properties associated with access to a palatable diet on these outcomes All rats had ad libitum access to chow or chow with 2 hr access to a palatable food. Singly-housed male Wistar rats were exposed to 3 consecutive days of episodic stress on postnatal days (PD) 27-29, while control rats received daily handling. Rats were tested in the social interaction test on PDs 63-67. Following sacrifice, the PFC and NAc were collected and dopamine receptor expression was determined via qPCR. Access to a palatable diet was associated with D_2 receptor downregulation in the PFC. The combination of stressor exposure and palatable diet results in an upregulation of D_1 and D_2 receptors in the NAc. While the functional significance of this upregulation is unknown, further work incorporating behavioral assays investigating cross-sensitization to drugs of abuse may provide interesting revelations.

Keywords: Juvenile stress, Anxiety, Obesity, High fat diet, Social Isolation; Dopamine Receptor; Prefrontal Cortex; Nucleus Accumbens; Reward

Introduction

In both humans and rodents, stress can shift food preferences towards a palatable diet, particularly a diet consisting of a high carbohydrate, sugar and fat content. Ingestion of palatable food amid a stressful environment has been associated with decreased adrenocorticotropic hormone (ACTH), corticosterone and a reduction in corticotrophin releasing factor (CRF) mRNA expression in the PVN among adult rats (Christiansen, Herman, & Ulrich-Lai, 2011; Dallman et al., 2003a; Pecoraro et al., 2004; Ulrich-Lai et al., 2007; Zeeni et al., 2012), indicating that a palatable diet may alter HPA axis response to stress. More recently, there has been evidence to suggest that the stress-reducing effects of a palatable diet are germane to early-life and juvenility. Indeed, access to a high fat cafeteria diet or chocolate cookies in rats reversed anxiety- and depressive-like traits induced by early life stressors (e.g. prolonged maternal separation), as well as normalized CRF expression and basal corticosterone in adulthood (Krolow et al., 2013b; Machado et al., 2013; Maniam & Morris, 2010a). We have also demonstrated that daily limited access to palatable diet in rats mitigated the long-term consequences of a sub-chronic juvenile stressor exposure (Mackay et al., 2014). In this study, rats with access to chow only displayed an anxiogenic profile in the social interaction test and elevated plus maze (EPM) that was normalized with access to a palatable diet. In addition, access to a palatable food was associated with lower basal corticosterone levels regardless of stressor exposure, which furthers the notion that palatable food can mitigate effects of juvenile stress in adulthood.

Even though the consumption of palatable food can ameliorate stressor-induced behavioral and physiological effects, it may also have long-term detrimental metabolic consequences. Whereas access to a palatable diet in our most recent work limited stress-induced outcomes in

adulthood, it was also associated with indices of metabolic syndrome, including increased adiposity, redistribution of fat pads with an emphasis on visceral obesity, as well as a trend towards glucose intolerance among stressed rats (Mackay et al., 2014). Consumption of a palatable diet also leads to increased plasma total cholesterol, reduced caloric efficiency and insulin resistance (Aschbacher et al., 2014; Dallman et al., 2005; Fachin et al., 2008; Pecoraro et al., 2004). The interplay between stress and diet may be particularly relevant to the period of adolescence as childhood and adolescent obesity has doubled in the last two decades and is now considered a global health issue (World Health Organization, 2015; World Obesity, 2014). Furthermore, childhood and adolescence can be a stressful time that involves a number of novel and unexpected experiences, as well as developmental changes (Murray et al., 2011).

Given evidence of the stress-reducing properties of a high fat/high sugar diet, the consumption of palatable food has been proposed as a method of self-medication against stress (Dallman et al., 2005; Meye & Adan, 2014). Interestingly, it has been suggested that the mechanism(s) providing stress relief by palatable food consumption likely resides at the intersection of metabolic, reward and stress regulatory circuits (Ulrich-Lai et al., 2010). One possible mechanism through which palatable foods exert their stress-reducing effects is through the modification the dopamine signaling within the mesolimbic 'reward' pathway, including dopaminergic afferents from the ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC) and hippocampus which all play a role in stress-induced palatable feeding (Meye & Adan, 2014). Brain structures within this pathway, particularly the PFC, NAc and hippocampus undergo critical re-organization and development during juvenility in both humans and rodents (Spear, 2000; Tsoory et al., 2007). Alterations in dopamine (DA) systems are also evident (Spear, 2000). With that said, these continuously developing structures are

vulnerable to stressors, potentiating changes that can increase the risk of psychopathologies like anxiety later on in adulthood (Avital & Richter-Levin, 2005; Mackay et al., 2014; Maslova, Bulygina, & Markel, 2002; Romeo et al., 2004; Tsoory et al., 2007; Tsoory & Richter-Levin, 2006).

The present study was conducted in order to further elucidate the long-term impacts of juvenile stress on DA receptor expression in the NAc and PFC and the potential stress-reducing properties associated with access to a palatable diet on these outcomes. Specifically, given that dopaminergic pathways are under development during adolescence and that palatable foods may exert their stress-reducing properties through hedonic mechanisms, we evaluated dopamine receptor expression changes in brain regions implicated in reward and stress. A secondary objective was to evaluate the potential metabolic outcome/impact of long-term access to a palatable diet following a juvenile stressor.

Materials and Methods

Animals. Forty 21 day old male Wistar rats obtained from Charles River (Quebec, Canada) were singly housed in standard plastic cages (37cm x 26 cm x 18cm) with bedding at a room temperature of 22±1°C on a 12 hour light-dark cycle (lights on at 0700hrs). Weight gain was measured on PDs 21, 30, 40, 50, 60, and 70. All procedures met the guidelines established by the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Ottawa Institute of Mental Health Research.

Experimental Design. An overview of the stress procedure and testing schedule is presented in Figure 21. Upon arrival, rats were randomly assigned to one of four conditions: (1) Chow + No Stress; (2) Chow + Stress; (3) Palatable + No Stress; and (4) Palatable + Stress. All animals underwent all behavioral tests.

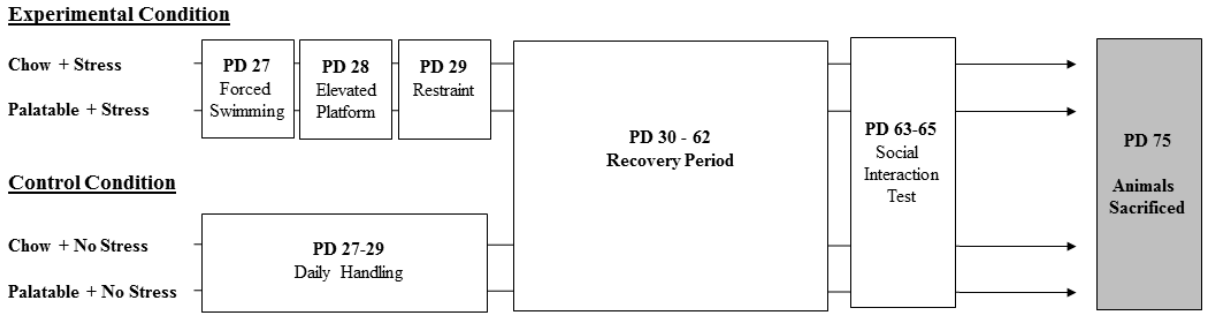

Figure 21. Summary of study design. Animals assigned to stress condition were exposed to a different stressor per day from PD 27-29 while control animals received daily handling. Animals were tested in adulthood following a recovery period. Brain tissue was collected at PD 75.

Diet. All rats were given ad libitum access to standard laboratory chow (3.4 kcal/g, 4.5% fat, 18.1% protein, 57.3% carbohydrate, Charles River Rodent Diet 5075, Agribrand Purina Canada, Woodstock, Ontario, Canada) and rats assigned to the palatable food condition were given limited daily access to a 45%Kcal Fat diet (4.7 Kcal/g, 23.2% fat, 17.3% protein, 47.6% carbohydrates, TD.08811, Harlan Laboratories, Madison, Wisconsin, USA). Palatable food was given daily at 8:00 and removed at 10:00 and intake was measured by subtracting the remaining amount from the initial weight. On PDs 21, 30, 40, 50, and 60, chow consumption was measured by subtracting the weight of the chow in the cage from its weight 24-hours earlier. Total caloric intake was calculated by multiplying the energy content (Kcal/g) of individual diets by the amount consumed and summing them. Calories from the palatable diet were calculated by multiplying the amount of food consumed by the energy content of the diet. Food intake data was collected from PD 21 to PD60.

Juvenile Stress Paradigm. The juvenile stress procedure used was a modification of the 3 day procedure described by Jacobson-Pick and Ritcher-Levin (2010). This procedure comprised 3 consecutive days of exposure to a different stressor per day throughout PD27-29.

PD 27 - Forced swimming. Rats were individually placed in a circular water basin (diameter 48cm; height 42cm; water depth 29 cm and a temperature of 22±2°C) for 10 min, during which they swam or floated continuously.

PD 28 – Elevated platform. Rats were individually placed on a small elevated platform (12cm x 12cm; 70cm elevation from water level) for three separate 30 min sessions. Platforms stood within a basin filled with water for the animals' protection should they fall off. During the intersession interval of 90 min rats were returned to their home cage. Animals that fell during the testing session were immediately returned to the platform for the remainder of the test session.

PD 29 – Restraint. Rats were placed for 30 min in a plastic restraining bag that prevented side-to-side movement and limited forward-backward movement. The plastic bag had a hole at the end closest to the rat's nose to allow for ventilation.

Behavioral Testing. Behavioral testing was conducted under low illumination (30 - 40 lux) between 10:00-13:00 daily following a 1 hr. period during which rats habituated to the testing room. Behavior was monitored via a video camera mounted above the arena.

Social interaction. The social interaction test was conducted over a total duration of three days. On habituation day 1 (PD 63), rats were matched to a partner from the same Diet x Stress condition (but from a different cage) on the basis of body weight, such that members of a pair did not differ by >10g. Paired rats were then placed in the arena (60 x 60 cm; 30cm high walls) together for five minutes. On habituation day 2 (PD 64), all rats were individually placed in the arena for a period of three min. On test day (PD 65), the same pairs of rats used on habituation

day 1 were placed in the arena and social behaviors of both rats were observed for 7 min. Total time spent in social interaction (including sniffing, climbing over each other, following, allogrooming, and play fighting) was recorded and each individual analyzed separately. Decreases in social interaction in rodents may be reflective of an anxiogenic profile (File & Seth, 2003).

Tissue Processing. On PD 75, animals were sacrificed by rapid decapitation and brains were immediately extracted and placed on a stainless steel brain matrix positioned on an ice block with slots that were 0.5mm apart. Coronal brain sections were taken and NAc and PFC were dissected according to the brain atlas of Paxinos & Watson (Paxinos & Watson, 1998). Brain sections were immediately stored at -80°C for subsequent quantitative Polymerase Chain Reaction (qPCR) analysis. Brain extractions and dissections occurred in an RNase free environment prevent contamination and RNA degradation. Animal carcasses were stored at -80°C until fat pads weight analysis.

Adiposity. Carcasses were thawed at 4°C and fat pads hand dissected and weighed. The fat pads collected included: mesenteric, retroperitoneal, subcutaneous inguinal white fat, and inter-scapular brown fat.

Reverse Transcription quantitative Polymerase Chain Reaction (qPCR): Primer sequences selected for qPCR are summarized in Table 4. Brain tissue was submerged and homogenized in TriZol© and total ribonucleic acid (RNA) was extracted according to the manufacturer's instructions (Invitrogen, Burlington, ON, Canada). Briefly, chloroform was used to isolate the RNA and isopropanol precipitated the RNA using linear polyacrylamide as a co-precipitant. Resulting RNA pellet was then dissolved in 10µL of Tris-EDTA buffer. Total RNA concentrations and quality were analyzed using the Thermo Scientific NanoDrop 2000

spectrophotometer (Thermo Scientific, Rockford, Illinois, USA) at an absorbance of 260nm. Total RNA was reverse-transcribed using the 5x iScript™ RT Supermix (Bio-Rad Laboratories, Hercules California, USA). Nuclease free water was added to adjust to a total volume of 20uL for each sample and samples were run on Bio-Rad T100 Thermal Cycler (Bio Rad Laboratories, Hercules California, USA) at 25°C for 5 minutes, 42°C for 30 minutes and 85°C for 5 minutes and then stored at -20°C.

Afterwards, 4uL aliquot of sample cDNA along with 5uL of Sso Advanced Universal™ SYBR© Green Supermix (Bio-Rad Laboratories, Hercules California, USA), 0.4uL of forward and reverse primer and 0.2uL of nuclease-free water were plated and analyzed in simultaneous quantitative polymerase chain reaction (qPCR). All samples, as well as non-template controls (all reagents mentioned above replacing complementary deoxyribonucleic acid (cDNA) with 4uL of nuclease free water) were run in duplicate. Plates were run on CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules California, USA) using the following thermal cycler program: initial denaturation at 95°C for 30 seconds, secondary denaturation at 95°C for 10 seconds and annealing at optimal primer specific temperature for 30 seconds. Secondary denaturation and annealing were repeated 39 times. Final elongation starting at 65°C for 5 seconds with a rise in temperature 0.5°C per cycle ramping at a rate of 0.5°C/s for 60 times. Primers that amplify β-actin (Actβ) and ribosomal protein L-19 (RPL19) were used as reference genes and were validated for their stability under the stress and diet conditions for this study. Reference genes were utilized for gene expression normalization such that the mean cycle quantification value (C_Q) of the two reference genes were subtracted from the C_Q of the target gene. Calculation of the mRNA fold changes using the $2^{-\Delta\Delta CQ}$ method (Livak & Schmittgen, 2001) then compared gene expression with treatment groups (stress or diet) relative to the control

chow fed group. Minimum information for publication of reverse transcription qPCR experiments (MIQE) guidelines were followed such that selected primers were optimized for annealing temperatures and a standard curve was conducted. Using a gradient of temperatures, an optimal range of annealing temperatures for the primer was selected for each tissue type. Afterwards, a standard curve was run to find the appropriate dilution factor for the tissue samples using the optimal annealing temperature. Primer efficiency and the cycle threshold were determined from the slope in relation to the absolute copy of RNA quantity and the C_Q values using Bio-Rad Amplification CFX Manager™ Software version 3.0. All primer pairs had a minimum efficiency of 90%.

Table 4

Primer sequences for genes of interest selected for reverse transcription quantitative polymerase chain reaction.

Gene		**Primer Sequence**
Actβ	Forward	5'TAT GCC AAC ACA GTG CTG TCT GG 3'
	Reverse	5'TAC TCC TGC TTC CTG ATC CAC AT 3'
RPL19	Forward	5' TGC AGC CAT GAG TAT GCT TAG 3'
	Reverse	5' GAG AGT TGG CAT TGG CGA TT 3'
D_1	Forward	5' GGT CCA AGG TGA CCA ACT TCT 3'
	Reverse	5' CCC AGA TGT TAC AAA AGG GAC C 3'
D_2	Forward	5' AAG ACA CCA CTC AAG GGC AAC 3'
	Reverse	5' ATC CAT TCT CCG CCT GTT CAC 3'
D_3	Forward	5' AGT CTG GAA TTT CAG CCG CA 3'
	Reverse	5' ACC GCT GTG TAC CTG TCT ATG 3'

Note. Actβ = β-actin; RPL19 = ribosomal protein L-19; D_1 = dopamine receptor 1; D_2 = dopamine receptor 2; D_3 = dopamine receptor 3.

Statistical analyses. Data obtained from the social interaction test, DA receptor mRNA expression in the NAc and PFC, and individual fat pad weight were analyzed by 2-way analysis of variance (ANOVAs) where Diet (2 levels; chow vs palatable food) and Stress (2 levels; stress vs. no stress) were the between group factors. Body weight was analyzed using 3-way repeated-measures ANOVAs, where Diet (2 levels) and Stress (2 levels) were the between group variables and Time (6 points) the repeated within group variable. Total caloric intake was analyzed using 3-way repeated-measures ANOVAs, where Diet (2 levels) and Stress (2 levels) were the between group variables and Time (8 points) the repeated within group variable. Calories from the palatable diet was analyzed using 2-way repeated-measures ANOVAs, where Stress (2 levels) was the between group variable and Time (8 points) the repeated within group variable.

Subsequent follow-up comparisons were conducted using *t* tests with a Bonferroni correction to maintain the alpha level at .05. For some measures a priori predictions that access to the palatable diet would alter the impact of the stressor were made. Analyses of the simple effects for interactions and a priori predictions were conducted irrespective of whether the F value for an interaction reached significance (Winer, 1962), especially when visual inspection of graphed results suggested that a significant difference may exist. Data points ±3 standard deviations from calculated means were considered as outliers and not included in statistical analysis (Taylor, 1997). Some rats were removed from the statistical analysis of variable due to missing data, thus the N and df associated with these measures vary across outcomes.

Results

Effect of stress and diet on weight and food intake. Body weight gain is depicted in Figure 22A. Repeated measures ANOVA revealed a significant Diet x Time interaction for weight, $F_{Diet\ x\ Time}(5, 180) = 3.56$, $p < .01$. No Stress x Time effect was observed, $F_{Stress\ x\ Time}(5,$

180) = .468, p = .800, and the interaction between Stress x Diet x Time was non-significant, $F_{Stress\ x\ Diet\ x\ Time}(5, 180) = .070$, p = .997. As expected, all rats gained weight over time and inspection of graphed results suggested that previously stressed rats with access to the palatable diet more weight relative to stressed rats with access to chow only; however, analysis of the simple effects comprising this interaction indicated no significant differences in weight between diets.

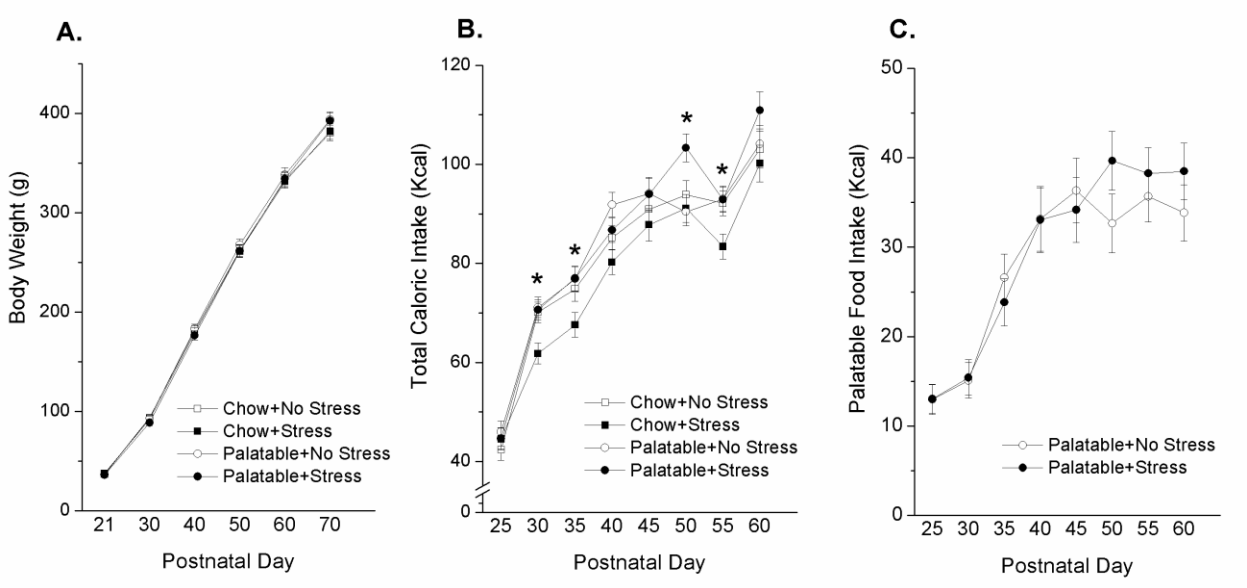

Figure 22. Effect of juvenile stress and diet on body weight and feeding behavior. A reduction in caloric intake relative to non-stressed controls was observed among rats with access to Chow only on PDs 30, 35 and 55. Juvenile stress did not impact body weight and palatable food intake. Data represents mean ± SEM. * Significant effect of Stress at $p < .05$.

Figure 22B depicts total caloric intake over the course of the study. A significant effect of Stress x Time, $F_{Stress\ x\ Time}(7, 252) = 3.90$, $p < .001$, and Stress x Diet x Time interaction was

observed in the total amount of calories consumed, $F_{Stress\ x\ Diet\ x\ Time}(7, 252) = 2.69$, $p = .011$. No effects of Diet x Time were observed, $F_{Diet\ x\ Time}(7, 252) = .588$, $p = .765$. Analyses of the simple effects comprising the Stress x Diet x Time interaction indicate that Chow+Stress rats consumed fewer calories relative to their non-stressed controls on PD 30, 35 and 55. A significant effect of Stress was observed in the palatable food group on PD 50, where stressed rats consumed more calories relative to their non-stressed controls.

Palatable food intake is depicted in Figure 22C. No significant differences in palatable food intake were observed, $F_{Stress\ x\ Time}(7,126) = 1.49$, $p = .177$.

Effect of stress and diet on anxiety-like behavior. Two-way ANOVA revealed a significant effect of the Stress x Diet interaction on total time spent engaging in social behaviors, $F_{Stress\ x\ Diet}(3, 35) = 9.53$, $p = .004$; Figure 23A. A significant main effect of Stress was also observed, $F_{Stress}(3, 35) = 7.75$, $p = .009$, and the main effect of Diet approached significance, $F_{Diet}(3, 35) = 3.84$, $p = .058$. Analyses of simple effect that comprised the Stress x Diet interaction indicated that exposure to juvenile stress was associated with reduced time in social interaction relative to non-stressed controls; however, this pattern was not observed among rats with access to the palatable diet. No significant main effect of Stress and Diet on number of squares crossed was observed, $F_{Stress}(3,35) = 1.02$, $p = .320$ and $F_{Diet}(3,35) = 2.85$, $p = .100$, respectively. The interaction between Stress and Diet was non-significant, $F_{Stress\ x\ Diet}(3,35) = .854$, $p = .362$; Figure 23B. Thus observed difference in social behaviors are not attributable to differences in locomotor behavior.

Time spent engaging in each individual social behavior are depicted in Figure 23C and D. A significant main effect for Stress and Diet was observed in time spent sniffing their partner, $F_{Stress}(3, 35) = 5.76$, $p = .022$ and $F_{Diet}(3, 35) = 10.70$, $p = .002$, respectively. The interaction

between Stress x Diet was also significant, $F_{Stress\ x\ Diet}(3, 35) = 7.54$, $p = .009$. While no main effect of Stress, $F_{Stress}\ (3, 35) = 2.99$, $p = .092$, and Diet, $F_{Diet}(3, 35) = 1.73$, $p = 1.97$, were observed in time spent moving over/under their partner, a significant interaction between Stress x Diet was observed, $F_{Stress\ x\ Diet}\ (3, 35) = 8.32$, $p = .007$. Follow-up comparisons analysing the simple effects that comprising the Stress x Diet interaction for time spent sniffing and time spent moving over/under their partner indicated that previous exposure to juvenile stress was associated with a reduction in these behaviors relative to non-stressed controls. This pattern was only observed among rats with access to Chow only. A significant Stress effect was observed in time spent allogrooming, whereby previous exposure to juvenile stress was associated with reduced allogrooming relative to non-stressed controls, $F_{Stress}(3, 35) = 5.77$, $p = .022$; Figure 23D. The main effect of Diet, $F_{Diet}(3, 35) = .570$, $p = .455$, and interaction between Stress x Diet, $F_{Stress\ x\ Diet}(3, 35) = 2.98$, $p = .093$, were non-significant for allogrooming. No significant effect of Diet and Stress was observed in time spent play fighting, $F_{Stress}\ (3, 35) = 2.86$, $p = .100$, $F_{Diet}\ (3, 35) = .206$, $p = .653$, and $F_{Stress\ x\ Diet}\ (3, 35) = .229$, $p = .635$. No significant effect of Stress and Diet was observed in time spent following their partner, $F_{Stress}\ (3, 35) = 2.60$, $p = .116$, $F_{Diet}\ (3, 35) = 2.42$, $p = .129$, and $F_{Stress\ x\ Diet}\ (3, 35) = 2.69$, $p = .110$; However, follow-up comparisons for these variables were completed based on visual inspection of the graph. Analysis of simple effects comprising the interaction between Stress and Diet indicated that stressed rats with access to chow only displayed reduced following behavior relative to their non-stressed controls. This pattern was not observed among rats with access to the palatable diet, as seen in Figure 23D.

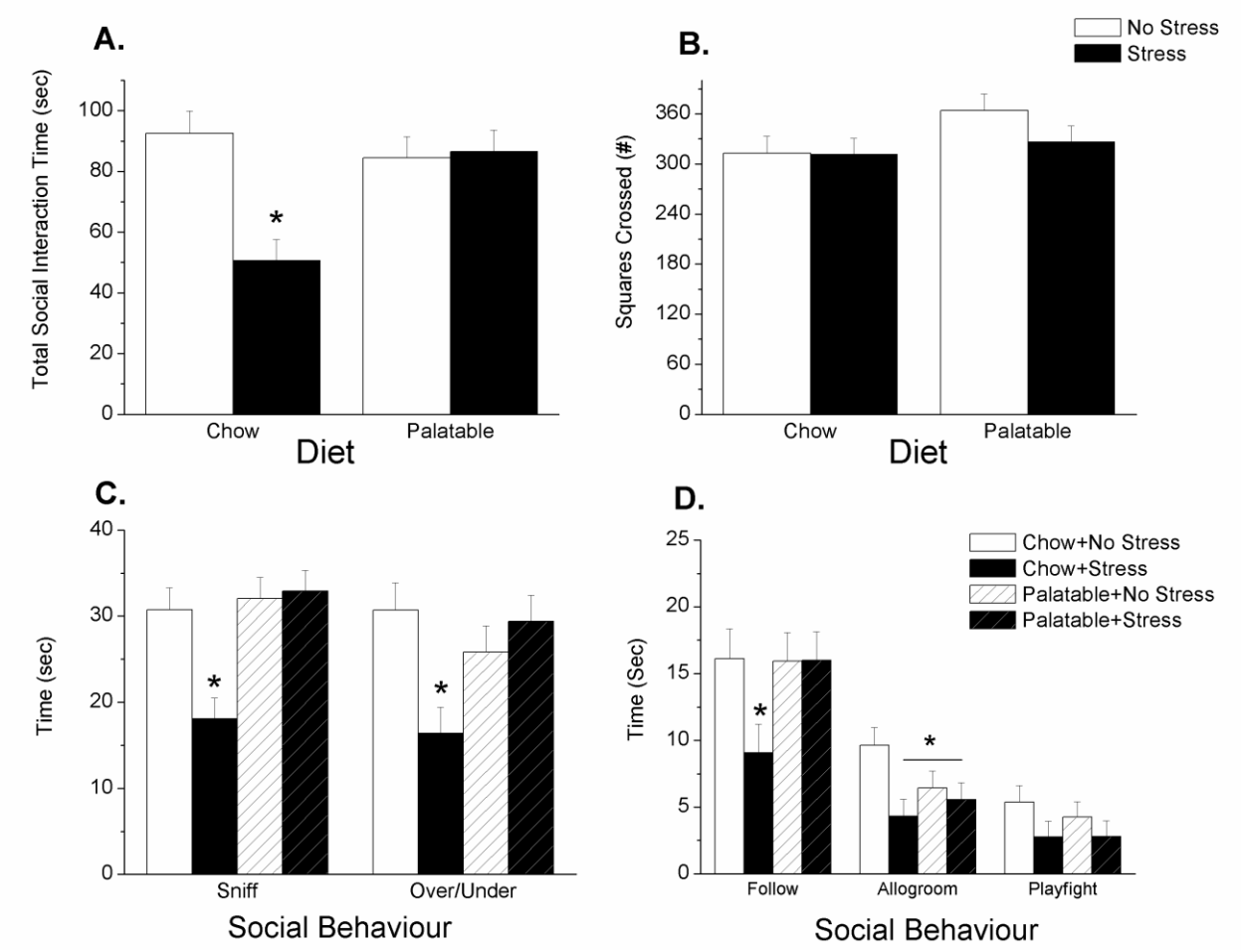

Figure 23. Effect of juvenile stress and diet on anxiety-like behavior. Exposure to juvenile stress was associated with an overall reduction in social behaviors relative to non-stress controls. Interestingly, this pattern was only seen among rats with access to Chow only. Data represents mean ± SEM. * Significant effect of Stress at $p < .05$.

Effect of stress and diet on adiposity. Two-way ANOVA revealed a significant Stress x Diet interaction on the total weight of dissected fat pads, $F_{Stress\ x\ Diet}(1,35) = 7.42$, $p = .010$; Figure 24A. A main effect for Diet, $F_{Diet}(1,35) = 5.02$, $p = .032$, was also observed, and the main effect for Stress was non-significant, $F_{Stress}(1,35) = 1.51$, $p = .228$. Follow-up analysis of the

simple effect comprising this interaction indicated that among rats with access to the palatable diet previous exposure to juvenile stress was associated with an increased in total fat pad weight relative to non-stressed controls.

Individual fat pad weights are depicted in Figure 24B. Two-way ANOVA revealed a significant interaction between Stress and Diet in the weight of subcutaneous inguinal fat pad, $F_{Stress\ x\ Diet}$ (1,35) = 6.99, p = .012. The main effects for Stress and Diet were non-significant, F_{Stress}(1,35) = 1.96, p = .170, and F_{Diet} (1,35) = 1.68, p = .203, respectively. Follow-up comparisons of the simple effects comprising the Stress x Diet interaction revealed a similar pattern observed in the total sum of fat pads. That it, previous exposure to juvenile stress was associated in an increased in subcutaneous inguinal fat pad weight relative to controls, but only among rats with access to the palatable diet. A significant Diet effect was observed in the weight of inter-scapular brown, F_{Diet}(1,35) = 13.75, p = .001; however the main effect for Stress, F_{Stress}(1,35) = .619, p = .437, and Stress x Diet interaction, $F_{Stress\ x\ Diet}$(1,35) = .022, p = .884, were non-significant. Similarly, a significant main effect of Diet was observed in retroperitoneal fat pad weight, F_{Diet}(1,35) = 5.66, p = .023. The main effect of Stress and interaction between Stress x Diet were non-significant, F_{Stress}(1,35) = 1.43, p = .240 and $F_{Stress\ x\ Diet}$(1,35) = 3.16, p = .084, respectively. Follow-up comparisons of the simple effects comprising the interaction between Stress and Diet on retroperitoneal fat pad weight were completed based on visual inspection of the graph. As depicted in Figure 24B, a significant increase in the weight of the retroperitoneal fat pad was observed among previously stressed rats with access to the palatable diet relative to their non-stressed controls. In contrast, chow-fed rats did not significantly differ from their non-stressed controls. No significant main effects of Stress and Diet were observed in the weight of the mesenteric fat pad, F_{Stress}(1,35) = .360, p = .533 and F_{Diet}(1,35) = 2.51, p =

.122, respectively; however, the interaction between Stress x Diet approached significance, $F_{Stress\ x\ Diet}(1,35) = 3.88$, $p = .057$. Follow-up simple effects analyses were completed based on visual inspection of the graphs and indicated that stressed rats with access to the palatable diet displayed increased mesenteric fat pad weight relative to their chow-fed counterparts.

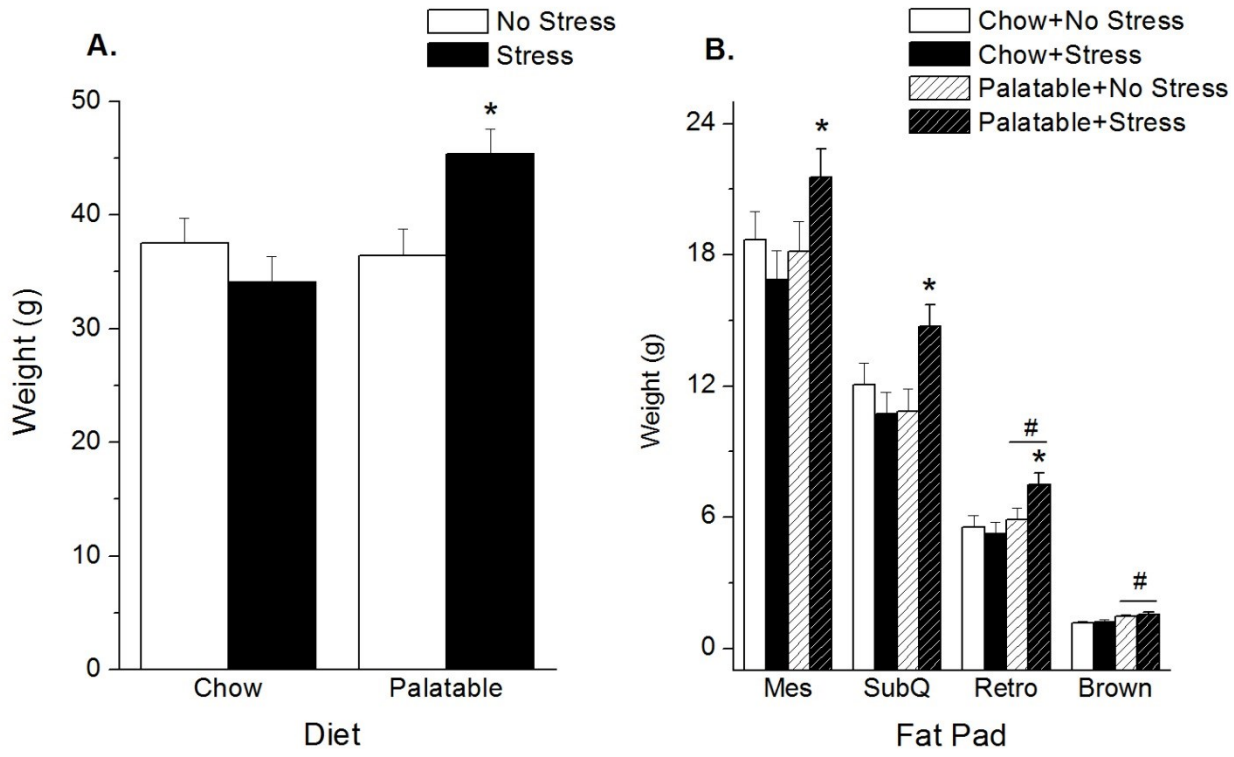

Figure 24. Impact of juvenile stress and diet on adiposity. Previous exposure to juvenile stress was associated with an increase in total fat pad weight (A), as well as an increase in mesenteric, retroperitoneal, and subcutaneous inguinal fat pads (B); however, this increase was only observed when rats were given access to the palatable diet. Mes = Mesenteric fat pad. Retro = retroperitoneal fat pad. Brown = Inter-scapular brown fat. Sub = Subcutaneous inguinal white fat pad. Data represents mean ± SEM. * Significant effect of Stress at $p < .05$; # Significant effect of Diet at $p < .05$.

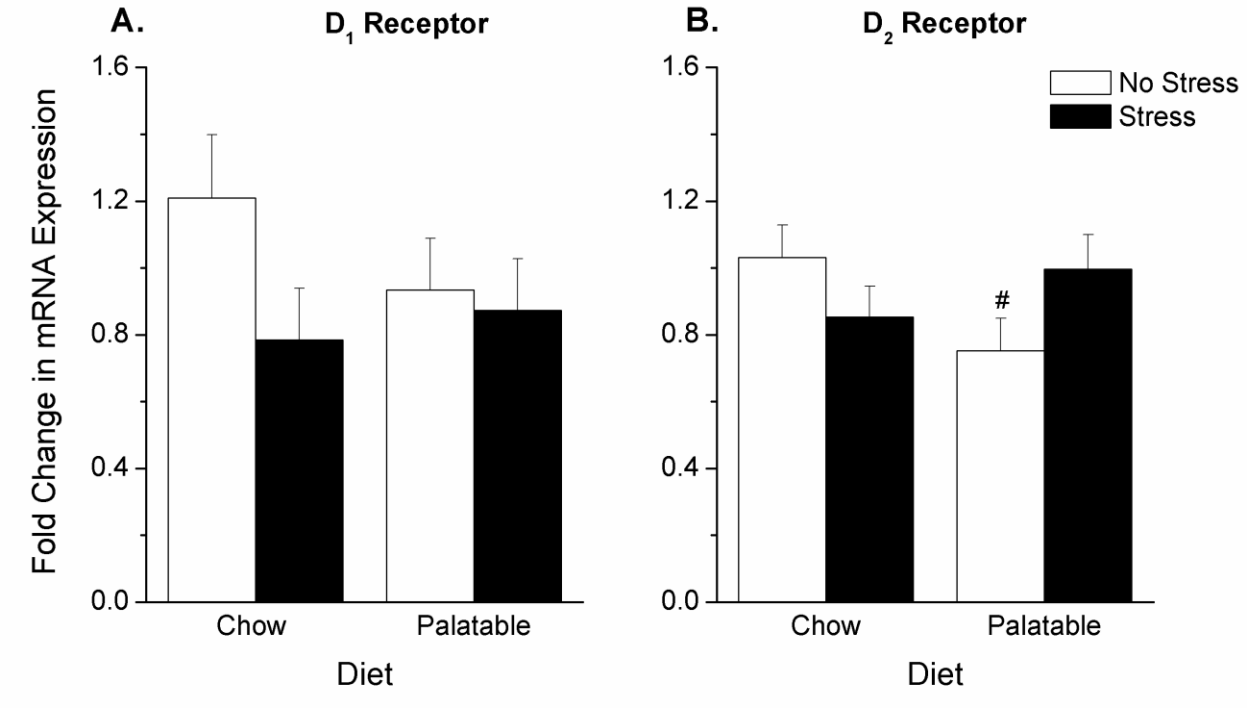

Figure 25. Impact of juvenile stress and diet on fold changes in mRNA expression of dopamine receptors in the mPFC. Among non-stressed controls, a downregulation of the D_2 receptor in the PFC was observed when rats were given access to the palatable diet. Data represents mean ± SEM. # Significant effect of Diet at $p < .05$.

Effect of stress and diet on dopamine receptor expression. In the mPFC, no significant main effects of Stress and Diet were observed in D_1 expression in the PFC, $F_{Stress}(1,29) = 2.20$, $p = .149$, and $F_{Diet}(1,29) = .325$, $p = .573$, respectively. The interaction between Stress and Diet was also non-significant, $F_{Stress\ x\ Diet}(1,29) = 1.24$, $p = .275$; Figure 25A. Two-way ANOVA revealed a significant interaction between Stress x Diet in D_2 mRNA expression, $F(1,32) = 4.63$ $p = .039$; Figure 25B. The main effects of Stress and Diet were non-significant, $F_{Stress}(1,29) = .118$, $p = .733$, and $F_{Diet}(1,29) = .485$, $p = .491$, respectively. Simple effect analysis comprising

the Stress x Diet interaction indicated a down-regulation of the D_2 expression amongst non-stressed rats with access to the palatable diet relative to chow-fed non stressed controls.

Dopamine receptor expression in the NAc is depicted in Figure 26. In the NAc a significant main effect of Stress, $F_{Stress}(1,21) = 6.70$, $p = .017$and Stress x Diet interaction, $F_{Stress\ x\ Diet}(1,21) = 4.99$, $p = .037$; Figure 26A.was observed in D_1 expression. No main effects of Diet were observed, $F_{Diet}(1,21) = .239$, $p = .630$. Simple effects analysis comprising the Stress x Diet interaction indicated that previous exposure to juvenile stress was associated with a significant up-regulation in D_1 expression relative to non-stressed controls but only when rats were given access to the palatable diet. A significant Stress effect was observed in D_2 expression, $F_{Stress}(1,21) = 4.22$ $p = .050$; Figure 26B. No main effect of Diet was observed, $F_{Stress}(1,21) = .004$ $p = .948$, and in the interaction between Stress and Diet was non-significant, $F_{Stress\ x\ Diet}(1,21) = 3.60$ $p = .072$. Follow-up simple effects analyses comprising the Stress x Diet interaction were completed based on visual inspection on the graph. Results indicated that previous exposure to juvenile stress was associated with a significant up-regulation in D_2 expression relative to non-stressed controls but only when rats were given access to the palatable diet. No significant effects of Stress and Diet on D_3 expression were observed in NAc, $F_{Stress}(1,21) = 2.19$ $p = .154$ and $F_{Diet}(1,21) = .004$ $p = .948$, respectively. The interaction between Stress x Diet was also non-significant, $F_{Stress\ x\ Diet}(1,21) = .009$ $p = .924$; Figure 26C.

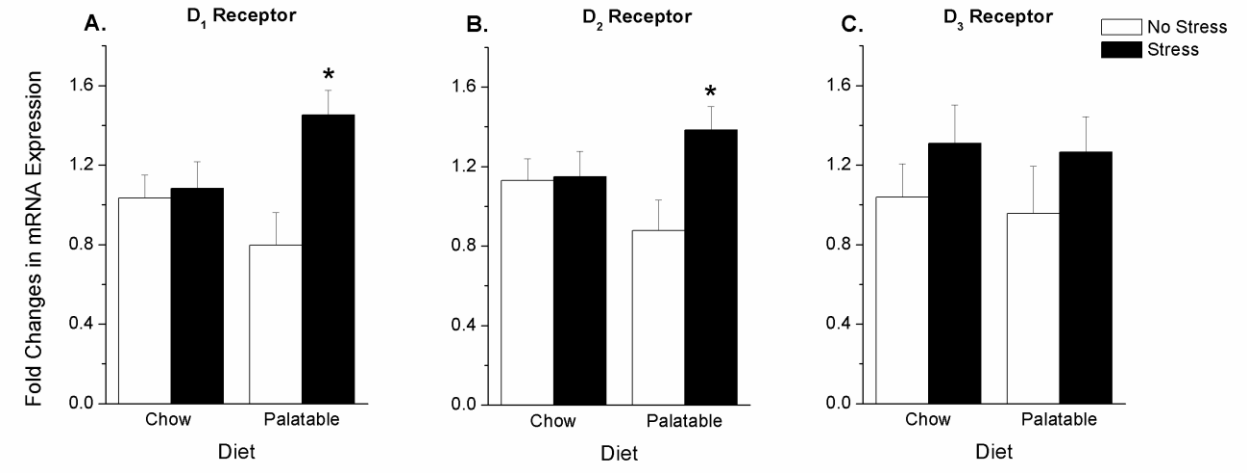

Figure 26. Impact of juvenile stress and diet on fold changes in mRNA expression of dopamine receptors in the NAc. Among rats with access to the palatable diet, previous exposure to juvenile stress was associated with an up-regulation of D_1 and D_2 receptor expression relative to non-stressed controls. Data represents mean ± SEM. * Significant effect of Stress at $p < .05$.

Discussion

The present study provides further evidence that stress experienced during juvenility can have long lasting effects on anxiety-like behavior in adulthood and that palatable foods may mitigate these negative impacts. We have previously demonstrated among group-housed animals that access to a palatable diet can mitigate the long-term effects of juvenile stress in the social interaction test (Mackay et al., 2014). Consistent with our previous results, exposure to a juvenile stress in singly-housed animals reduced social behaviors, an indication of increased anxiety, which was normalized by consumption of a palatable diet. Similar stress-buffering effects of palatable foods have been documented in response to stressors applied in adulthood (Buwalda et al., 2001; Christiansen et al., 2011; Dess, 1992; Fachin et al., 2008; Foster et al., 2009; Kant & Bauman, 1993; la Fleur et al., 2005; Pecoraro et al., 2004; Ulrich-Lai et al., 2007; Ulrich-Lai et

al., 2010; Ulrich-Lai et al., 2011; Young, 2000) and the post-natal period (Maniam & Morris, 2010a), so the results of the present study provide further evidence that access to a palatable diet may protect against the long-term behavioral consequences of stressor exposure.

Interestingly, no effect of juvenile stress on the palatable food intake was observed in the present study, which is in keeping with our previously reported results using group-housed animals (Mackay et al., 2014). Stressor exposure has been associated with a shift in preference towards a palatable diet among rodents provided a choice between a standard laboratory diet and a palatable alternative (Chuang et al., 2011; Dallman, 2010; Dallman et al., 2005; Pecoraro et al., 2004; Ulrich-Lai et al., 2011). A possible contributing factor to this consistent lack of palatable food preference observed in our studies is that rats were only afforded limited access to the palatable diet during the light cycle. Studies documenting shifts in preference towards a palatable diet have generally offered rodents a choice of a variety of nutrient sources that are provided ad libitum (Foster et al., 2009; la Fleur et al., 2005; Maniam & Morris, 2010a; Maniam & Morris, 2010b; Pecoraro et al., 2004). Furthermore the relative delivery of nutrients has varied as some studies have provided rats with access to commercial snacks available from grocery stores (Maniam & Morris, 2010a; Maniam & Morris, 2010b), while others have provided individual allocations of each nutrient source (e.g. fat, protein, carbohydrates) (Dallman, 2010; Dallman et al., 2005; Pecoraro et al., 2004) or water sweetened with sugars (Foster et al., 2009; Ulrich-Lai et al., 2007; Ulrich-Lai et al., 2011). Interestingly, there is some evidence to suggest that preference for a sweetened drink develops following stressor exposure, even when rats are afforded limited access to the sweetened drink (Ulrich-Lai et al., 2007; Ulrich-Lai et al., 2011), suggesting that the relative delivery (e.g. pellet vs. liquid) of the palatable diet may matter.

Despite the potential stress buffering effects of access to a palatable diet on anxiety-like behaviors, present metabolic results would appear to suggest that this mitigating effect came at a cost. No differences in body weight were observed; however stressed rats with access to the palatable diet displayed a significant increase in total fat pad weight relative to those that had not been stressed. Analysis of the individual fat pads revealed that these rats also displayed a significant increase in mesenteric, retroperitoneal and sub-cutaneous fat pads relative to their non-stressed controls. We have previously demonstrated a selective increase in mesenteric fat pad weight among group-housed rats that had access to high-fat/high-sugar diet and were exposed to the same 3-day sub-chronic stressor in juvenility (Mackay et al., 2014). Our results in pair housed and single housed animals are consistent with previous evidence suggesting that chronic stress promotes visceral fat accumulation, as well as increased weight gain and glucose intolerance (Kyrou, Chrousos, & Tsigos, 2006; Kyrou & Tsigos, 2009; Yau & Potenza, 2013).For example, exposure to chronic juvenile stress in mice promotes the accumulation of adipose tissue in visceral depots relative to subcutaneous storage (Schmidt et al., 2009) and social isolation between PD 21-28 has associated with increased abdominal adiposity (Arcego et al., 2014).

In addition to limiting anxiety-like behaviors and enhancing adiposity in juvenile stressed-rats, limited access to a palatable diet over the 18-weeks of the present study was associated with changes in DA receptor mRNA expression in two brain regions that have been implicated in both stress and reward. A downregulation of D_2 receptors in the PFC amongst non-stressed rats with access to the palatable was observed, an effect that was not apparent in non-stressed rats with access to Chow only. No changes in D_1 or D_2 mRNA expression in the NAc were observed among non-stressed rats with access to the palatable diet. Access to a diet high in fat and sugar alone has

been associated with changes in DA receptor expression at various sites within the mesolimbic circuit (Alsio et al., 2010; Alsio et al., 2014; Ong, Wanasuria, Lin, Hiscock, & Muhlhausler, 2013; Vucetic, Carlin, Totoki, & Reyes, 2012); however the majority of studies investigating the impact of a palatable diet alone on dopamine receptor expression have provided unrestricted access to the palatable diet. Consistent with the results of the current study, mice given access to a 60% fat diet for 12-weeks also display reduced D_2 mRNA expression in the PFC, as well decreased DA levels (Carlin, Hill-Smith, Lucki, & Reyes, 2013); however, there are few studies investigating DA receptor expression in the PFC. In contrast to the present results, a study using a similar diet to the present study (45%Kcal Fat diet) demonstrated that chronic unrestricted access to a palatable diet was associated with reductions in D_1 and D_2 expression in the NAc (Alsio et al., 2010). Interestingly, downregulation of these receptors in the NAc in this study was also shown to be independent of excessive calorie consumption, as well as body weight gain, leading the authors to suggest that it is the ingestion of the high fat/high sugar food itself that leads to the observed changes in DA receptor expression (Alsio et al., 2010). However, there appears to be variability within the literature regarding the impact of a palatable diet on D_2 expression in the NAc. For example, in one study where individually housed rats were given *ad libitum* access to a palatable diet for 6 weeks, there were no significant differences in D_2 expression within the NAc (Ong et al., 2013). D_2 receptor expression in the NAc was also unchanged following 20-weeks of exposure to high-fat diet (Vucetic et al., 2012). It is possible that D_2 expression in the NAc is initially altered following exposure to palatable diet and expression is gradually restored over time (Ong et al., 2013). Downregulation of D_2 has also been reported in the VTA (Vucetic et al., 2012), as well as the brainstem (Alsio et al., 2014) following unrestricted access to a high fat diet. While there is evidence to suggest that access to a palatable

diet can impact dopamine related gene expression, the results of the present study are not directly comparable given the restricted access to the palatable food. Although, the present results suggest that 18-weeks of daily limited access to a palatable diet starting in juvenility is sufficient to alter PFC D_2 receptor mRNA expression in adulthood.

Exposure to juvenile stress was associated with an upregulation of D_1 and D_2 receptors in the NAc; however, this pattern was only observed when rats were given access to the palatable diet. The functional significance of this upregulation is not known. As already mentioned, access to a high fat and a high sucrose diet has typically been associated with reduced expression of D_1 and/or D_2 receptors in the mesolimbic pathway (Alsio et al., 2009; Kovalenko et al., 2014; Ong et al., 2013). Surprisingly however, no changes in D_1 or D_2 mRNA expression at the NAc from palatable food consumption were observed in unstressed rats. There is some evidence that stressor (or stress hormone) exposure can influence DA receptor mRNA expression at the NAc. From example, chronic treatment with corticosterone during adolescence in rats that were previously exposed to neonatal stress, increased D_2 receptor expression at the NAc (Hill et al., 2014) and a prenatal stressor exposure also elicited an increase in D_2 receptor expression at the NAc in adulthood (Lakehayli et al., 2015; Said et al., 2015). Interestingly, chronic cocaine administration upregulated D_1 receptor mRNA expression at the NAc, a finding that coincided with the manifestation of cocaine induced behavioral sensitization (Unterwald, Ho, Rubenfeld, & Kreek, 1994). It is possible that the present results also provide some evidence of cross-sensitization, whereby previous exposure to juvenile stress may increase the neural response to a natural food reward. Palatable foods have been suggested to have similar neural effects as drugs of abuse and have been associated with cross-sensitization to low-doses of amphetamine (Avena & Hoebel, 2003). Indeed, there is mounting evidence to support cross-sensitization to drugs of abuse following

exposure to stress in juvenility. From a behavioral perspective, exposure to non-habituating stressors in adolescence, such as the chronic variable stress CVS has been associated with cross-sensitization to cocaine induced locomotion when animals are tested in the adulthood (Lepsch et al., 2005). Cross-sensitization towards drugs of abuse is proposed to relate to the sensitization of mesolimbic dopamine system following juvenile stress exposure (Burke & Miczek, 2014). Stress during adolescent may results in immediate sensitization of the DA system, as exposure to early adolescent stress has been associated with increase DA content in the NAc following an injection of amphetamine in mid-adolescents (Cruz, Marin, Leao, & Planeta, 2012). Interestingly, adult stress in this study was not associated with an increased in DA content in the NAc (Cruz et al., 2012). There is also some evidence to suggest that behavioral cross-sensitization mat not directly correlate with changes in DA system, and that alterations in DA release may be specific to the location of study (e.g. NAc core vs shell) (Burke & Miczek, 2014; Burke, Renner, Forster, & Watt, 2010). While the present study provides some evidence that stress and diet induced some specific changes in D_1 and D_2 receptor expression in the NAc, further information regarding DA content or locomotor-behavior following administration of drugs of abuse would provide some further clarification of apparent changes in DA system functionality.

In summary, the present study provides further evidence that exposure to a sub-chronic juveniles stressor can influence anxiety-like behaviors in adulthood. Taken together with our previously reported results in group-housed animal (Mackay et al., 2014) we have also demonstrated additional evidence that access to a palatable diet can mitigate these behavioral consequences, at the cost of increased adiposity. Further clarification is needed to determine the functional significant of D_1 and D_2 receptor upregulation in the NAc observed among stressed rats with access to the palatable diet.

Disclosure

All of the authors declare that they have no direct or indirect conflicts of interests of a financial nature relevant to the present work.

Acknowledgements

This project was funded by an operating grant from the Canadian Institute for Health Research (CIHR) awarded to Zul Merali, and by a CIHR doctoral Canada Graduate Scholarship awarded to J. Christine MacKay. Technical contributions of Mohammed Abu Nada are gratefully acknowledged.

Chapter 7: General Discussion

The level of perceived stress experienced by individuals is reported to be continuously rising (Cohen & Janicki-Deverts, 2012), highlighting the importance of further understanding the long-term implications of stressor exposure. Stress has been identified as an important precipitating factor in the development and maintenance of numerous psychiatric disorders including anxiety and mood disorders, as well as psychotic disorders (Heim & Nemeroff, 1999; Kendler et al., 1999; van et al., 2008). To date, the majority of animal research on the neurobiological consequences of stressor exposure has focused on the prenatal, early postnatal and adult periods. Until more recently, the adolescent period has been largely uninvestigated, despite evidence in the human literature suggesting that stress during this developmental window may be associated with depression, drug use and other psychological issues in adulthood (Compas et al., 1995; Heim & Nemeroff, 1999; Kendler et al., 1999). Furthermore, the period of adolescence is marked by substantial remodelling of stress-sensitive brain regions related to emotional and learning processes such as the prefrontal cortex, hippocampus, and the amygdala (Spear, 2000), highlighting the need for a more thorough understanding of the long-term implications of stressor exposure during this transitional period of development. How an individual chooses to cope with a stressor can influence the potential consequences of stressor exposure (Compas et al., 2001). Studies investigating the impact of stressors in adulthood have begun to highlight the use of palatable foods as a form self-medication against the negative consequences of stressors (Dallman et al., 2005). This type of coping strategy may be of particular relevance to the adolescent time period given the increase in stressors experienced during this time, the associated developmental changes in the brain, as well as the reported increase in childhood and adolescent obesity. As such, the present dissertation sought to further

characterize the short and long term effects of stress exposure during juvenility, through the investigation of both a social and non-social stressor. A secondary objective was to elucidate the potential stress buffering effects of access to palatable foods, as well as how stress and diet influence reward circuitry.

The impact of stressors applied in adulthood: Lessons learned from the resident intruder paradigm

The first study of this dissertation investigated the immediate and delayed effects of social defeat on behavioral indices of depression and anxiety among adult rats. No significant immediate or delayed effects of social defeat were apparent in this study, which is in contrast to some of the reported effects of social defeat on both anxiety- and depressive-like behaviors and stress-reactivity (Berton et al., 1998; Bhatnagar & Vining, 2003; Bhatnagar et al., 2006; Hollis et al., 2010; Koolhaas et al., 1997; Meerlo et al., 1996; Patki et al., 2013; Ruis et al., 1999; Rygula et al., 2005). Some of the discrepant findings could be attributable to several methodological differences, and which may have impacted the results of Chapter 2. Of particular relevance is the inconsistent aggressive behaviors observed among residents, which also included residents who chose to isolate themselves from the intruder rats during interactions resulting in no social contact between the resident and intruder. One of the largest influencing factors was the lack of resident screening, which was prompted by limited access to retired breeders through our animal supplier. Previous work has highlighted the need for screening and priming of aggressive behaviors in the resident rat in order to optimize the paradigm (Koolhaas et al., 2013). The potential stress inducing effect of the resident/intruder model may also warrant attention in this study given the long duration (21 days) and exposure to multiple intruder rats per day. Indeed, resident rats have been reported to habituate to defeat encounters (Patki et al., 2013), however

the unpredictable and chronic nature of the resident/intruder exposure employed may have led to "burnout" among some residents. Communications with another laboratory employing this paradigm suggested that this may be a commonly encountered challenge when residents are overused and not afforded adequate rest time between defeat sessions (B. Bingham, personal communication, May 31, 2011). More favourable results may have been observed with consistently aggressive residents which would have been facilitated by increased sample size, as well as adequate screening measures to identify residents with aggressive tendencies. Thus the first study in this dissertation imparted some valuable lessons concerning the effective application of the resident/intruder paradigm as a model of social stress.

The impact of stressors applied in adolescence

Review of the literature concerning long-term impact of stressors revealed a relative paucity of studies focusing on the adolescent period relative to studies focusing on the adult or early post-natal periods. There is evidence supporting the idea that brain functioning and development in juvenility is unique and distinct from adulthood as well the early postnatal period, suggesting the need to explore potentially distinct effects that stress may have during this stage of development. Relative to the early postnatal period, development of the HPA axis is thought to be reaching a developmental asymptote (Spear, 2000); however, the HPA axis functioning is reported to be more reactive and sensitive to stressors which may be attributable to a delayed negative feedback response (Jankord et al., 2011; Spear, 2000). As such, juvenile rats exhibit higher or prolonged ACTH and corticosterone release compared to adults (McCormick & Mathews, 2010). This period of development is also marked by an overproduction and pruning of synaptic connections across a variety of brain regions that are involved in the neurobiological response to stressors such as the PFC, hippocampus, and amygdala (Spear, 2000; Tsoory et al.,

2007). On-going development of various neurotransmitter systems is also underway during the adolescent period (Burke & Miczek, 2014; Spear, 2000). These neurobiological differences, coupled with the fact that adolescence is associated with marked increase in stressor perception/exposure (Compas et al., 2001), highlights the relevance of investigating the impact of stress during this developmental window.

Social stressors. The second study in this dissertation marked a shift in focus towards the juvenile period due to its proposed sensitivity to the effects of stress. An ethologically relevant stressor, namely the resident intruder paradigm, was selected in order to better capture the potential effect of social stress during the adolescent period, as adolescence is highlighted by increased sociability both in humans and rodents (Panksepp, 1981; Spear, 2000). As such, it has been proposed that the majority of stressors encountered by adolescents may involve a social component (Buwalda et al., 2011). In Chapter 3, exposure to juvenile social defeat was associated with anxiogenic outcome in adults, as reflected in the open field test. No significant effects of defeat were observed in the elevated plus maze or the forced swim test. Reduced rate of weight gain amongst defeated rats, coupled with open field results, suggest that the experience may have been stressful as this is a well-established consequence of stressor exposure in rats (Marti et al., 1996). Indeed, there appears to be some evidence to suggest that exposure to social defeat may have long term implications into adulthood (Vidal et al., 2007; Vidal et al., 2011a; Vidal et al., 2011b); however the observed effects, as well as methodology across is studies are inconsistent (Bourke & Neigh, 2011; Buwalda et al., 2013; Hayashida et al., 2010; Watt et al., 2009; Weathington et al., 2012), suggesting caution in the interpretation of the reported findings.

Contrary to some previous reports (Vidal et al., 2007; Vidal et al., 2011b; Watt et al., 2009), juvenile social defeat was associated with an apparent increase in sociability which was

most pronounced among rats with access to chow only. We hypothesized that this may be related to previous exposure to social contact and cues among rats which were exposed to the residents. Juvenility in rodents has been proposed to be a crucial developmental window during which social play and contact plays an important role in the development of socially competent adults (Einon & Morgan, 1977). Studies investigating the impact of long-term social isolation suggest that deprivation of social contact is associated with behavioral and social deficits in adulthood (Pellis & Pellis, 2007). Interestingly, brief 1-hour exposure to a juvenile peer protected against social deficits in adulthood among isolates (Einon et al., 1978). Exposure to play behaviors appears to be of paramount importance as neither segregated pair-housing (e.g. wire mesh) or housing with a non-playful adult female alleviates the social deficits in adulthood (Einon et al., 1978; Pellis et al., 1999). It is possible that exposure to the resident/intruder model may have served as a substitute for juvenile social play, which in turn made defeated rats appear more socially competent when tested in the social interaction test. This may have been further impacted by how the social interaction test was conducted, as rats were paired with a partner from the same experimental group. As such, all rats with previous social experience were paired with one another which resulted in the comparison of socially-competent pairs to control rats that had experienced long-term social isolation. While single housing was necessary in order to bolster the effects of defeat (Bingham et al., 2011), it would have been of interest to evaluate social behavior towards a novel conspecific rat. This would provide information concerning how social defeat may impact the initiation of social contact.

There are several factors to consider in the interpretation of how juvenile social defeat may impact functioning in adulthood. Review of the literature would seem to suggest that the developmental timing of defeat exposure may impact the long-term behavioral results.

Adolescence in the rat can be sub-divided into three main time periods: pre-pubescent/early adolescence (PD 21-34); mid-adolescence/pubertal onset (PD 34-46); and late adolescence (PD46-59) (McCormick & Green, 2013). The large majority of studies reporting long-term consequences of juvenile social defeat involved exposing the juvenile to the resident during mid-adolescence. Indeed, exposure to social defeat during mid- and late-adolescence was associated with increased immobility in the FST (Bourke & Neigh, 2011; Weathington et al., 2012), social avoidance (Vidal et al., 2007; Vidal et al., 2011b), deceased sucrose preference (Bourke & Neigh, 2011), and increased anxiety in the EPM (Weathington et al., 2012). Exposure to social defeat during early adolescence increased defensive burying in adulthood in one study (Bingham et al., 2011), however minimal negative effects have been reported in others (Buwalda et al., 2013). Housing conditions also appear to be of importance, as paired housing during juvenile social defeat is associated with reduced anxiety-like behavior and increased locomotion in adulthood (Watt et al., 2009). Social isolation has been proposed to be a necessary component in the detrimental effect of social defeat (Ruis et al., 1999), and pair-housing conditions may serve as a protective factor against the potential negative consequences (Isovich, Engelmann, Landgraf, & Fuchs, 2001; Ruis et al., 1999).

Of additional consideration is the nature of the resident intruder paradigm itself. The methodology of juvenile social defeat bears a close resemblance to it adult counterpart, as the sole modification is a shift in the developmental age of the intruder rat. In our laboratory, priming sexually-mature residents to display aggressive behavior towards the sexually-immature juvenile counterparts was challenging. Indeed, only a small number of resident rats screened demonstrated sufficient aggression to be included in the study, and aggressive behaviors varied as the study progressed. The majority of resident rats appeared to display reduced aggression

over the course of the study and some residents needed to be removed due to their lack of consistency. Our observations appeared to be consistent with those of other laboratories (B. Bingham, personal communication, May 31, 2011). Indeed, the application of a social stressor limits the level of control afford to the investigator. For example, in contrast to applying a restraint stress for a fixed duration using a specific apparatus, it is far more challenging to ensure that each intruder receives the same and consistent "dose" of social defeat, as each resident/intruder interaction is unique which introduces variability into the model. While a level of standardization may be introduced through the application of established screening criteria, there is no way to predict how each individual interaction will unfold, leading to a potential variability in social defeat doses. This uncertainty is not exclusive to juvenile social defeat, and was observed to be a source of variability within Chapter 2 as well. However, one could also argue that this variability may better simulate the phenomenon of bullying among humans, as each bully-victim dyad would also be unique. It is noteworthy that given the large developmental gap between the resident and intruder, use of this paradigm in juveniles may be best conceptualized as a model of childhood physical abuse, which may be qualitatively different from bullying by same aged-peers.

Non-social stressors. The remaining collection of studies provides further evidence that exposure to a sub-chronic non-social stressor in juvenility is associated with long-term behavioral and neurochemical consequences in adulthood. Results also appear to be independent of housing conditions. In Chapter 4, no immediate effects of juvenile stress were observed when rats were test 24 hours following the termination of stressors; however, protracted effects were observed when rats were tested in adulthood (Mackay et al., 2014). An anxiogenic profile was observed in both the EPM and social interaction tests. A similar profile was observed in studies

reported in both Chapters 5 and 6, among singly housed rats. This 3-day model, originally developed by Jacobson-Pick and Richter-Levin (2010), has been previously associated with a long-term increase in anxiety-like behavior in both the EPM and open field tests (Jacobson-Pick & Richter-Levin, 2010; Jacobson-Pick & Richter-Levin, 2012). Furthermore, elevated basal corticosterone levels (Ilin & Richter-Levin, 2009) and altered GABA receptor expression in the amygdala and hippocampus (Ilin & Richter-Levin, 2009) have also been reported. Other juvenile stress protocols involving similar methodologies (e.g. elevated platform stress, and other 3-day stressor combinations) have also demonstrated a consistent association between juvenile stressor exposure and anxiety-like behavior in adulthood (Avital et al., 2006; Avital & Richter-Levin, 2005; Brydges et al., 2012; Horovitz et al., 2014; Tsoory et al., 2007).

The observation that early-life exposure to stressors is associated with increased risk of adult psychopathology is thought, in part, to be related to the negative impact of stress-related hormones, such as corticosterone (cortisol in humans) (Duman, 2002; Kasckow, Lupien, Behan, Welge, & Hauger, 2001; Lee, Ogle, & Sapolsky, 2002). Indeed, the adolescent brain is uniquely sensitive to the impact of stress-hormones, as the neural circuity underlying cognitive and emotional processing are under development during this time period (Spear, 2000). Furthermore, brain regions associated with this circuitry are rich in GC receptors (McEwen, 2012). For example, among humans, GR mRNA in the prefrontal cortex was elevated in adolescence in comparison to early childhood and young adulthood (Perlman, Webster, Herman, Kleinman, & Weickert, 2007). Taken together, these observations demonstrate that the brain may be sensitive to the impact of GCs in an age-dependent manner (Lupien, McEwen, Gunnar, & Heim, 2009). As such, exposure to stressors during different developmental windows would translate into different outcomes. As stated previously, juveniles display a prolonged HPA axis response to

stress (McCormick & Mathews, 2010; Vazquez & Akil, 1993), as well as potentiated release of both ACTH and corticosterone in response to repeated stress which may relate to an immaturity of the GC negative feedback system (Jankord et al., 2011; Spear, 2000).

In Chapter 5, previous exposure to juvenile stress was associated with elevated basal corticosterone, as well as an exaggerated HPA-axis activity following exposure to a novel stressor, suggesting that juvenile stress may have resulted in the sensitization of the HPA-axis. However, these results are somewhat inconsistent with our findings from the group-housed animals in Chapter 4, where no differences in basal corticosterone was observed between chow-fed rats exposed to juvenile stress and their controls. Similarly, no significant effect of juvenile stress on basal corticosterone was observed among singly housed animals in Chapter 6. It is noteworthy that this discrepancy may be attributable to variations in measurement as corticosterone in each study may have been sampled at different times. Evidence indicates that corticosterone levels vary according to the circadian cycle (Atkinson & Waddell, 1997), and may further complicate the comparison of basal levels across chapters. The increased sensitivity of the HPA axis may be more meaningful consequence of juvenile stress relative to basal (or unchallenged) differences. As such, it is possible that differences may have been evident in Chapters 4 and 6 had we completed an HPA axis challenge employing a novel stressor, for instance. However, it is noteworthy that the literature on the enduring effects of juvenile stressors on adult HPA function and reactivity is quite variable. Some studies investigating various non-social stressors suggest that, when tested in adulthood, male rats exposed to juvenile stress demonstrate increased basal corticosterone (Pohl, Olmstead, Wynne-Edwards, Harkness, & Menard, 2007; Schmidt et al., 2007; Sterlemann et al., 2008; Uys et al., 2006a; Uys et al., 2006b), as well increased HPA axis reactivity to a novel stressor (Isgor, Kabbaj, Akil, & Watson,

2004). When the literature is often collapsed across a variety of well-established stressors, including non-social (e.g. foot shock, restraint, elevated platform, swim stress) and social stressors (e.g. social instability model, isolation, resident intruder), the large majority of studies report no enduring impact of juvenile stress on HPA axis markers and reactivity (Isgor et al., 2004; Maslova et al., 2002; Mathews, Mills, & McCormick, 2008; McCormick et al., 2005; McCormick et al., 2008; Overmier & Murison, 1991; Toledo-Rodriguez & Sandi, 2007). This apparent discrepancy may be attributable to the apparent paucity of studies investigating this time point; however, preliminary patterns in the literature would seem to suggest that a history of stress in juvenility does not translate into definitive deficits in HPA function in adulthood. It is possible that previous exposure to juvenile stress influenced the HPA axis vulnerability to subsequent exposure to stress in adulthood (McCormick, Mathews, Thomas, & Waters, 2010). Increased exposure to stressors may account for the observed increased in basal corticosterone in Chapter 5, as these rats underwent chronic social isolation. Relative to those in Chapter 6, they also underwent more behavioral testing (e.g. EPM and FST) which may have been as stressful experience.

Interestingly, FST results between Chapters 4 and 5 were inconsistent, as a significant effect was stress was solely observed in Chapter 5. As such, rats exposed to juvenile stress in this study displayed increased immobility relative to their controls. Effects of juvenile non-social stressors on depressive-like behaviors in adulthood are somewhat inconsistent. The majority of studies incorporating behavioral measures of depressive-like behaviors have employed a CVS protocol which may not be directly comparable to the 3-day sub-chronic stressor employed in the present set of studies. Results across studies have been mixed, with some reporting increase immobility (Wilkin et al., 2012) and others reporting no discernable effects of CVS (Toth et al.,

2008; Wilkin et al., 2012). Observed discrepancies between Chapters 4 and 5 may be attributed to the compounding effects of long term social isolation resulting from single housing conditions; however the impact of social isolation on behaviors in the FST is variable, with some studies reporting impairments in the FST (Brenes et al., 2008), and other reporting no impact (Hall et al., 1998; Hall et al., 2001; Hong et al., 2012); However, it is noteworthy that results of Chapter 5 highlighted some important considerations regarding the FST protocol that was employed in previous chapters, which may have confounded results. The initial FST protocols (15-18 cm water depth) allowed for rats to have contact with the bottom of the testing apparatus and levels of immobility were reported to be high (Porsolt et al., 1978); However, this testing protocol was demonstrated to be unreliable in detecting the antidepressant properties of selective-serotonin-reuptake-inhibitors (SSRIs) (Cryan et al., 2002; Cryan et al., 2005; Lucki, 1997). Modifications to the testing protocol to increase water depth to 30 cm revealed a change in behavior in the test, where increased water depth facilitated more active behaviors, such as climbing and swimming (Abel, 1994; Slattery & Cryan, 2012). This modification also offered more reliable detection of the antidepressant properties of SSRIs (Detke & Lucki, 1996; Lucki, 1997). For the present set of studies, Chapters 2-4 utilized the same FST testing apparatus (20 x 45 cm cylinder) and water depth of 30 cm. While the original testing apparatus and water depth used was consistent with modified FST protocols cited in the literature (Abel, 1994; Bogdanova et al., 2013), observation of subjects in earlier studies suggested that the water depth may be insufficient as larger adult rats may have been able to touch the bottom of tanks with their tails. We hypothesized that this was allowing rats to stabilize themselves with their tails, which may have confounded the test. The ability to touch the bottom of the tank appeared to be particularly problematic amongst rats with access to the palatable diet as they were generally much heavier

and larger than chow fed counterparts. As such, in order to mitigate potential confounding effects of water depth, the subsequent studies in Chapter 5 utilized an increased water depth (35 cm) and a different testing apparatus (18 x 60 cm). This modification did not allow rats to touch the bottom of the testing apparatus and potentially offered a more accurate picture of how juvenile stress and diet impact behavior in the FST; however, further studies are needed using the modified procedure in Chapter 5 in order to determine if these results can be replicated. It may also be interesting to consider the angle of floatation, as a wider angel of floatation (e.g. more vertical position within the tank) has recently been associated with decreased buoyancy and increased swimming (Chen, Faas, Ferando, & Mody, 2015).

The use of palatable foods as a means to cope with stress

Many individuals are familiar with the colloquial term "comfort eating" and most people can reflect back on a time where they may have consumed comforting food/snack in order to cope with a stressor. Within the literature, a stressor-induced preference for palatable foods (especially those with a high fat and/or sugar content) has been documented in both animals and humans (Dallman, 2010; Gibson, 2006; O'Connor et al., 2008; Zellner et al., 2006). While the majority of evidence supporting this phenomenon is in adults (Buwalda et al., 2001; Christiansen et al., 2011; Fachin et al., 2008; Foster et al., 2009; Kant & Bauman, 1993; la Fleur et al., 2005; Pecoraro et al., 2004; Ulrich-Lai et al., 2007; Ulrich-Lai et al., 2010; Ulrich-Lai et al., 2011; Young, 2000), there is growing evidence that access to palatable foods may serve to buffer against the long-term impact of maternal separation (Lee et al., 2014; Maniam & Morris, 2010a; Maniam & Morris, 2010b) and some physiologic effects of social isolation during early adolescence (Krolow et al., 2013b). This preliminary evidence, coupled with the suggestions that

adolescence represents a period of increased stress, suggests that palatable food may have some utility in the management of effects of stressor exposure during this developmental window.

Social stressors. Given the lack of apparent long-term behavioral impact of adult social defeat in Chapter 2, it was difficult to discern whether or not access to a palatable diet had stress reducing properties. However, there was modest evidence to suggest that access to a palatable diet may have had some stress-buffering effects following exposure to social defeat in juvenility. In Chapter 3, access to the palatable diet appeared to mitigate the anxiogenic effect revealed in the open field test. Furthermore, access to the palatable diet appeared to be protective against the stress-induced reduction of weight gain seen amongst the defeated chow-fed rats. However, of particular interest are the observed differences in behavior during the resident intruder interactions. Analysis of resident/intruder interactions suggests that access to the palatable diet may have imparted some degree of resilience within the interactions, as those with access to the palatable food spent less time in submissive posture and had an increased latency to separation from the resident. This observed difference raises some interesting questions regarding the potential latency between initial access to a palatable diet and onset of its stress-reducing effects. One could hypothesize that the stress reducing effects could have been apparent during initial defeat exposure and thus impact how social defeat stress may have been perceived or perhaps increasing the threshold required to result in negative consequences. As such, observed differences in adult could represent a carry-over effect from of initial buffering towards the initial "dose" of stress received.

A potential mechanism of action could reside in the HPA axis response to resident intruder interactions. Adolescence has been proposed to be a time of increased sensitivity to stress as pre-pubertal rats exhibit higher or prolonged ACTH and corticosterone release compared to adults

(McCormick & Mathews, 2010), and pre-pubescent males may not habituate to repeated restraint stress (Romeo et al., 2006). Access to a palatable diet dampened the HPA axis response to both acute and chronic stressors (Dallman et al., 2006; Foster et al., 2009; Levin, 1996; Maniam & Morris, 2010a; Maniam & Morris, 2010b; Strack et al., 1997; Zeeni et al., 2012). Investigation of basal corticosterone levels at sacrifice revealed no significant differences between the two juvenile stress groups, suggesting that diet did not have a major impact on HPA axis tone. However, this observation does not shed light on how diet may have impacted HPA axis reactivity in juvenility. One could hypothesize that access to a palatable diet may have attenuated HPA axis response to resident/intruder interactions, perhaps correcting for the observed heightened HPA axis response seen in adolescence. Corticosterone sampling during resident/intruder interactions may provide some interesting insights into whether or not the diet may have had immediate stress-buffering on HPA-axis reactivity. It would also be interesting to determine whether or not corticosterone levels are negatively correlated with measures of social defeat (e.g. time spent in submissive posture).

Interestingly, a significant diet effect in the FST was observed in Chapter 2, whereby all rats with access to the palatable diet displayed increase immobility regardless of whether or not they were exposed to social defeat in adulthood. This would seem to suggest that access to a palatable diet engendered a depressive profile and some studies have argued that consumption of a high fat/high sugar diet may be linked to an increased risk for depression. Furthermore, epidemiological research suggests that obesity is associated with an increased risk for developing depression (Dong, Sanchez, & Price, 2004; Zhao et al., 2009). Exaggerated HPA-axis function with corresponding depressive-like behavior in FST has been previously reported among non-stressed animals with free-access to a high-fat diet (Park et al., 2014; Soulis et al., 2007;

Tannenbaum et al., 1997). Consumption of a high fat diet in dams has also been associated with increased immobility in the FST when off-spring are tested in adulthood suggesting an increased susceptibility to depressive-like behaviors (Giriko et al., 2013). Access to a high-fat diet also exacerbated depressive-like behaviors in the Findler's Sensitive Line, a genetic model of depression (Abildgaard et al., 2011). In Chapter 2, access to the palatable diet and exposure to social defeat was associated with increased HPA-axis reactivity to a swim stressor. Coupled with behavioral results from the FST, results from Chapter 2 would seem to support the contention that consuming a high fat/high sugar diet may lead to a depressive phenotype; however, there are some methodological issues that warrant consideration. Levels of immobility were correlated with fat pad weight, and all rats with access to the palatable diet displayed significantly increased both body weight and adiposity. Body weight may have a significant effect on behavior in the FST (Bogdanova et al., 2013), which has prompted some researchers to statistically subtract the effects of body weight through multivariate analysis of covariance (Brenes et al., 2008). The previously mentioned concerns regarding the FST water level used in these studies also introduce an additional confound to the present results, as review of testing footage indicated that larger rats were able to prop themselves up with their tails, which may have facilitated immobility. While the potential confounding factors of body weight and water level cannot be discounted, the current evidence in the literature regarding the impact of high fat/high sugar diet would seem to suggest that access to the palatable diet may have altered depressive-like behaviors. However, replications of the present studies with an appropriate FST protocol are needed to provide further insight. The inclusion of other behavioral assays (e.g. sucrose preference test) investigating the anti-depressant properties of the palatable diet may also yield more information.

Non-social stressors. In general, access to a palatable diet appears to mitigate the long-terms consequences of exposure to a 3-day sub-chronic stressor in juvenility to a certain extent. In Chapter 4, access to a palatable diet mitigated the anxiogenic profile (in the EPM and social interaction test), as well as protected against the stress-induced reduction in the rate of weight gain amongst chow-fed rats. We observed a significant decrease in basal plasma corticosterone concentrations among rats with access to palatable food, which is consistent with previous reports that access to a palatable diet may dampen HPA axis activity (Foster et al., 2009; Pecoraro et al., 2004; Ulrich-Lai et al., 2007; Ulrich-Lai et al., 2010; Ulrich-Lai et al., 2011). In contrast to Chapter 4, we suggested in Chapter 5 that access to a palatable diet was not sufficient to mitigate the impact of the 3-day sub-chronic stressor when rats are additionally exposed to long-term social isolation associated with single housing conditions. In this regard, singly housed rats in Chapter 5 displayed increased anxiety-like behaviors in the EPM and SI, as well as a depressive profile in the FST. Given that long-term social isolation from early juvenility has been associated with increased anxiety-like (Chappell et al., 2013; Hall, 1998; Hall et al., 1998; Hall et al., 2000; Hellemans et al., 2004; Jankowska et al., 1991; Lodge & Lawrence, 2003; Maisonnette et al., 1993; Parker & Morinan, 1986; Weiss et al., 2004; Wright et al., 1991; Wright et al., 1991) and depressive-like behaviors (Brenes et al., 2008) in adulthood, we hypothesized that the observed results were the compounding effects of the application of a sub-chronic stressor amid the backdrop of the chronic stress of social isolation. Somewhat surprisingly, we were not able to replicate the reduced social behavior observed in Chapter 5, as the results of Chapter 6 demonstrated a very clear mitigating effect of the palatable diet in the social interaction test. While single-housing conditions were present in both studies, rats in Chapter 6 were only tested in the social interaction test. In contrast, rats in Chapter 5 were tested

in the EPM 24 hours prior to the start of the social interaction testing protocol. One could hypothesize that previous testing in the EPM constituted a stressor and that the effects of the EPM may have carried over into the social interaction teat. Indeed the EPM was developed as a behavioral assay to assess anxiety-like responses in rodents and draws on rodent's natural fear of heights and open spaces and preference for dark and enclosed areas (Walf & Frye, 2007). The discrepancy in results between Chapters 5 and 6 highlights another important methodological consideration which is the structuring of testing protocols to ensure that behavior in one test is not impacted by the prior test history. There is minimal research investigating the effects of study design when it comes to the relative timing of behavioral tests, although there is consensus that tests should be administered from least invasive to most invasive gradients. There is some evidence from mouse studies to suggest that some behavioral tests are sensitive to previous test measures (McIlwain, Merriweather, Yuva-Paylor, & Paylor, 2001), as well as evidence to suggest that mice benefit from a minimum of 1-2 days of rest between behavioral tests (Paylor, Spencer, Yuva-Paylor, & Pieke-Dahl, 2006).

Despite some variance across behavioral testing, we consistently demonstrated a stress-buffering effect of the palatable diet on HPA axis function and reactivity in studies employing a non-social juvenile stressor. A significant diet effect was observed in both Chapters 4 and 5 on basal corticosterone whereby rats with access to the palatable diet displayed reduced basal HPA axis tone. Furthermore, in Chapter 5, stressed rats with access to chow only displayed exaggerated HPA axis activity that carried forward following exposure to a novel air puff stressor. This pattern was not observed among stressed rats with access to the palatable diet, suggesting that the diet may have had a protective effect against the long-term changes in HPA axis functioning imparted by exposure to the juvenile stress. Taken together, our results

demonstrate that the HPA dampening effects of palatable foods reported in adulthood (Christiansen et al., 2011; Foster et al., 2009; Laugero et al., 2001; Pecoraro et al., 2004; Ulrich-Lai et al., 2007; Ulrich-Lai et al., 2010; Ulrich-Lai et al., 2011) can also be extended to the juvenile period. The apparent discrepancies between physiological and behavioral measures in Chapters 5 and 6 led us to hypothesize that the stress dampening effect of the palatable diet may have an effectiveness "threshold" which may be exceeded by the potential compounding effects of exposure to multiple stressors (e.g. sub-chronic 3-day stress, social isolation, behavioral testing). There is some evidence to suggest that the potential stress-buffering effectiveness of a palatable diet is associated with the rate and/or number of exposures. For example, when adult rats are given access to a 30% sucrose solution HPA axis dampening in response to a restraint stress is optimized through numerous brief exposures to limited amounts of sucrose drink (Ulrich-Lai et al., 2011). This brief exposure was found to be more effective than increasing the volume and duration of drink availability, and independent of the caloric value of the drink (Ulrich-Lai et al., 2011). While this type of exposure (e.g. 4mL, twice a day for 14 days) was associated with only modest reductions in HPA axis response to restraint, the authors hypothesized that the physiological impact may be cumulative over time (Ulrich-Lai et al., 2011). As such, it is possible that the "dose" of palatable diet used in Chapter 5 was insufficient to overcome the behavioral effect of compound stressors (e.g. social isolation, juvenile stress and behavioral testing). Alternatively, the stress-buffering effects of palatable diets on the behavioral outcomes may also be mediated through a different pathway(s). Further studies investigating the cumulative effects of stressor exposure and variable dosing regimens of palatable diet may provide further information concerning the potential effectiveness "threshold" of using food as a coping strategy.

Coping at a cost: The metabolic impact of access to palatable diet amid stressful environment

As expected, long-term access to a palatable diet was associated with negative health consequences across all studies. In general, rats with access to the palatable diet consistently weighed more than chow fed rats, which appeared to be independent of whether or not rats had limited access (Chapter 3-6) or free-access to the palatable diet (Chapter 2). We also demonstrated increased adiposity that appeared to be dependent on how adiposity was quantified. In Chapters 3 and 5, quantitative magnetic resonance spectroscopy (QMR) revealed no significant differences in total body fat, while notable differences were found when individual fat pads were hand dissected and weighed, as in Chapter 2, 4 and 6. QMR is a non-invasive technique that allows for the quantification of different types of soft tissues, such as lean mass, fat, free water and total water, by measuring the differential magnetic resonance properties of hydrogen atoms within tissues when a pulsed radio frequency is applied within a stable magnetic field (Nixon et al., 2010). QMR provides information regarding the relative body composition of subject, which allows for an estimation of total body fat. While QMR data are highly correlated with other well-established methods for determining total body fat (Wolden-Hanson, 2010), use of this technique in a sub-set of studies in this dissertation did not appear to offer the level of specificity needed in order to highlight the potential impacts of diet and stress on adiposity.

Using the hand dissection method (Chapters 2, 4 and 6), we consistently demonstrated that rats with access to the palatable diet displayed an increase in fat pad weights. In Chapter 4 and 6, previous exposure to a juvenile stress was associated with an apparent re-distribution of fat towards abdominal fat fads. This raises important questions when investigating the potential contribution of palatable diet in the development of metabolic syndromes, as total body fat

(TAT) is not uniformly distributed throughout the body. Accumulation of fat within specific depots is observed in obesity, including increase in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). Evidence suggests that there may be an increased risk for various negative metabolic outcomes, including accumulation of specific adipose pads (Kuo et al., 2007; Pou et al., 2009). Of particular interest is the observation that monitoring of VAT in humans studies is more closely correlated with poorer metabolic and pathological outcomes of obesity, relative to monitoring TAT or SAT (Fox et al., 2007; Weisberg et al., 2003). Furthermore, elevations in VAT are also associated with alterations in metabolic functioning that is characteristic of metabolic syndrome (Demerath et al., 2008; Despres, 2007).

The impact of access to the palatable diet on the ability to tolerate a glucose load revealed some interesting patterns. Across all studies, control rats with access to the palatable diet did not display glucose intolerance, which was somewhat unexpected given the evidence of negative metabolic impact of a diet high in fat and sugar. This seems somewhat inconsistent with previous reports of the associated between high-fat/high-sugar diets and the development of glucose intolerance (la Fleur, Luijendijk, van der Zwaal, Brans, & Adan, 2014; Packard, Ghosal, Herman, Woods, & Ulrich-Lai, 2014; Solomon, Jankord, Flak, & Herman, 2011; Woods, Seeley, Rushing, D'Alessio, & Tso, 2003); however, an important consideration is the relative access to the diet, as access to the palatable diet was restricted in Chapters 3-6. While glucose tolerance was not observed in Chapter 2, where rats were given ad libitum access, it is possible that the method of glucose administration (e.g. gastric gavage) may have had an impact on measured results. The results of Chapters 5 and 6 would seem to suggest that the addition of stress may alter the story. In Chapter 4, previously stressed rats with access to the palatable diet displayed a trend towards glucose intolerance. This observation reached clear statistical significance when

the study was repeated in singly-housed animals in Chapter 5.

Potential mechanism of action for stress-reducing effects of palatable foods

There has been growing interest in characterizing the potential underlying mechanism through which palatable foods may be exerting their effects. Recent work with stressors in adulthood would appear to suggest that the hedonic/rewarding properties of palatable foods may mediate the stress-buffering effects of palatable food via the brain's reward circuitry (Ulrich-Lai et al., 2010). In Chapter 6 we sought to characterize how juvenile stress and access to a palatable diet may alter DA receptor expression in the NAc and PFC, two areas that have been implicated in neural circuits related to reward and feeding. Exposure to the palatable diet on its own was associated with a downregulation of D_2 receptors, which is consistent with other reports of changes in DA receptor expression at various sites within the mesolimbic circuit (Alsio et al., 2010; Alsio et al., 2014; Ong et al., 2013; Vucetic et al., 2012); However, of particular interest was the associated changes in DA receptor expression among stressed rat with access to the palatable diet. In these rats, exposure to juvenile stress was associated with an upregulation of D_1 and D_2 receptors in the NAc. The functional significance of this upregulation is not known. We hypothesised that these results may provide some evidence of cross-sensitization, whereby previous exposure to juvenile stress may increase the neural response to a natural food reward. Future studies investigating DA content/utilization in the NAc or locomotor-behavior following administration of drugs of abuse would provide some further clarification of apparent changes in DA system functionality.

Summary

In summary, the present collection of studies provides further evidence that exposure to stress in juvenility is associated with long-term consequences on physiological and behavioral

consequences in adulthood. This is in keeping with the current animal and human literature suggesting that exposure to stress in adolescence predisposes an individual to the development of depressive- and anxiety-related symptoms in adulthood.

An important consideration arising from the present collection of studies is that of methodological consistency in both the execution of stressor and behavioral paradigms. This was particularly evident in the resident intruder paradigm, where variability in resident/intruder interactions may have resulted in an inconsistent dose of stress across intruders. Our FST results suggest that further investigation into the relationship between adiposity and immobility may provide some insight into the observed increase in depressive-like behaviors in rats with access to the palatable. Modification to the water levels used in FST testing, and incorporating other measures of behavioral despair are necessary to further elucidate the relationship between depressive-symptoms and a high-fat/high-sugar diet.

Most importantly, we have demonstrated that access to a palatable diet can mitigate some of the behavioral and physiological consequences of juvenile stress, at the cost of poor metabolic consequences. These findings are in line with observations in adulthood and the postnatal period. Although the functional significance is unknown, access to a palatable diet and previous exposure to a non-social stressor in juvenility was associated with changes in DA receptor expression in the NAc. Our results also provide some preliminary evidence that the relative stress-buffering effects of a palatable diet may have an effectiveness threshold that may depend of the "dose" of stress that the animal receives. Furthermore, the results of Chapter 3 appears to suggest that the timing of the introduction of the diet may matter, as access to a palatable diet prior to stress exposure may impart a degree of resilience. This highlights important

methodological considerations when designing future studies that aim to investigate the stress dampening effects of a palatable reward.

References

Abel, E. L. (1994). Behavioral and physiological effects of different water depths in the forced swim test. *Physiol.Behav., 56*, 411-414. http://dx.doi.org/10.1016/0031-9384(94)90215-1

Abelaira, H. M., Reus, G. Z., & Quevedo, J. (2013). Animal models as tools to study the pathophysiology of depression. *Rev.Bras.Psiquiatr., 35 Suppl 2*, S112-S120. http://dx.doi.org/10.1590/1516-4446-2013-1098

Abildgaard, A., Solskov, L., Volke, V., Harvey, B. H., Lund, S., & Wegener, G. (2011). A high-fat diet exacerbates depressive-like behavior in the Flinders Sensitive Line (FSL) rat, a genetic model of depression. *Psychoneuroendocrinology, 36*(5), 623-633. http://dx.doi.org/10.1016/j.psyneuen.2010.09.004

Aguilera, G. (1998). Corticotropin releasing hormone, receptor regulation and the stress response. *Trends Endocrinol.Metab., 9*, 329-336. http://dx.doi.org/10.1016/s1043-2760(98)00079-4

Aguilera, G., Pham, Q., & Rabadan-Diehl, C. (1994). Regulation of pituitary vasopressin receptors during chronic stress: Relationship to corticotroph responsiveness. *Journal of Neuroendocrinology, 6*, 299-304. http://dx.doi.org/10.1111/j.1365-2826.1994.tb00586.x

Akana, S. F., Dallman, M. F., Bradbury, M. J., Scribner, K. A., Strack, A. M., & Walker, C. D. (1992). Feedback and facilitation in the adrenocortical system: unmasking facilitation by

partial inhibition of the glucocorticoid response to prior stress. *Endocrinology, 131*(1), 57-68. http://dx.doi.org/10.1210/en.131.1.57

Akana, S. F., Hanson, E. S., Horsley, C. J., Strack, A. M., Bhatnagar, S., Bradbury, M. J. et al. (1996). Clamped Corticosterone (B) Reveals the Effect of Endogenous B on Both Facilitated Responsivity to Acute Restraint and Metabolic Responses to Chronic Stress. *Stress., 1*(1), 33-49. http://dx.doi.org/10.3109/10253899609001094

Akerfeldt, M. C., & Laybutt, D. R. (2011). Inhibition of Id1 Augments Insulin Secretion and Protects Against High-Fat Diet-Induced Glucose Intolerance. *Diabetes*. 60(10), 2506 - 2514. doi: http://dx.doi.org/10.2337/db11-0083

Akyol, A., McMullen, S., & Langley-Evans, S. C. (2011). Glucose intolerance associated with early-life exposure to maternal cafeteria feeding is dependent upon post-weaning diet. *British Journal of Nutrition,* 1-15. http://dx.doi.org/10.1017/s0007114511003916

Albeck, D. S., McKittrick, C. R., Blanchard, D. C., Blanchard, R. J., Nikulina, J., McEwen, B. S. et al. (1997). Chronic social stress alters levels of corticotropin-releasing factor and arginine vasopressin mRNA in rat brain. *Journal of Neuroscience, 17*(12), 4895-4903. http://www.jneurosci.org/

Alonso, R., Griebel, G., Pavone, G., Stemmelin, J., Le, F. G., & Soubrie, P. (2004). Blockade of CRF(1) or V(1b) receptors reverses stress-induced suppression of neurogenesis in a mouse model of depression. *Molecular Psychiatry, 9*(3), 278-86, 224. http://dx.doi.org/10.1038/sj.mp.4001464

Alsio, J., Olszewski, P. K., Norback, A. H., Gunnarsson, Z. E., Levine, A. S., Pickering, C. et al. (2010). Dopamine D1 receptor gene expression decreases in the nucleus accumbens upon long-term exposure to palatable food and differs depending on diet-induced obesity phenotype in rats. *Neuroscience, 171*(3), 779-787. http://dx.doi.org/10.1016/j.neuroscience.2010.09.046

Alsio, J., Rask-Andersen, M., Chavan, R. A., Olszewski, P. K., Levine, A. S., Fredriksson, R. et al. (2014). Exposure to a high-fat high-sugar diet causes strong up-regulation of proopiomelanocortin and differentially affects dopamine D1 and D2 receptor gene expression in the brainstem of rats. *Neuroscience Letters, 559*, 18-23. doi: 10.1016/j.neulet.2013.11.008

Alsio, J., Roman, E., Olszewski, P. K., Jonsson, P., Fredriksson, R., Levine, A. S. et al. (2009). Inverse association of high-fat diet preference and anxiety-like behavior: a putative role for urocortin 2. *Genes Brain Behav., 8*(2), 193-202. doi: 10.1111/j.1601-183X.2008.00464.x

American Psychological Association. (2014). *Stress in America: Are Teens Adopting Adutls' Stress Habits?* Retrieved from: http://www.apa.org/news/press/releases/stress/2013/stress-report.pdf

Anisman, H., & Matheson, K. (2005). Stress, depression, and anhedonia: caveats concerning animal models. . *Neuroscience & Biobehavioral Reviews, 29*(4-5), 525-546. doi: 10.1016/j.neubiorev.2005.03.007

Anisman, H., & Merali, Z. (1999). Understanding stress: characteristics and caveats. *Alcohol Research & Health, 23*(4), 241-249. http://pubs.niaaa.nih.gov/publications/arh23-4/241-249.pdf

Arcego, D. M., Krolow, R., Lampert, C., Noschang, C., Ferreira, A. G., Scherer, E. et al. (2014). Isolation during the prepubertal period associated with chronic access to palatable diets: effects on plasma lipid profile and liver oxidative stress. *Physiol Behav., 124*, 23-32. doi: 10.1016/j.physbeh.2013.10.029

Arcego, D. M., Krolow, R., Lampert, C., Noschang, C., Pettenuzzo, L. F., Marcolin, M. L. et al. (2013). Stress during the pre-pubertal period leads to long-term diet-dependent changes in anxiety-like behavior and in oxidative stress parameters in male adult rats. *Neurochemical Research, 38*(9), 1791-1800. doi:10.1007/s11064-013-1083-3

Armario, A., Luna, G., & Balasch, J. (1983). The effect of conspecifics on corticoadrenal response of rats to a novel environment. *Behav.Neural Biol., 37*(2), 332-337. http://dx.doi.org/10.1016/S0163-1047(83)91425-5

Asano, Y. (1986). Characteristics of open field behavior of Wistar and Sprague-Dawley rats. *Jikken Dobutsu, 35*(4), 505-508. Retrieved from PM:3803437

Aschbacher, K., Kornfeld, S., Picard, M., Puterman, E., Havel, P. J., Stanhope, K. et al. (2014). Chronic stress increases vulnerability to diet-related abdominal fat, oxidative stress, and metabolic risk. *Psychoneuroendocrinology, 46*, 14-22. doi: 10.1016/j.psyneuen.2014.04.003

Atkinson, H. C., & Waddell, B. J. (1997). Circadian variation in basal plasma corticosterone and adrenocorticotropin in the rat: sexual dimorphism and changes across the estrous cycle. *Endocrinology, 138*(9), 3842-3848. doi:10.1210/endo.138.9.5395

Avena, N. M., & Gold, M. S. (2011). Food and addiction - sugars, fats and hedonic overeating. *Addiction, 106*(7), 1214-1215. doi: 10.1111/j.1360-0443.2011.03373.x

Avena, N. M., & Hoebel, B. G. (2003). A diet promoting sugar dependency causes behavioral cross-sensitization to a low dose of amphetamine. *Neuroscience, 122*(1), 17-20. doi:S0306452203005025

Avital, A., Ram, E., Maayan, R., Weizman, A., & Richter-Levin, G. (2006). Effects of early-life stress on behavior and neurosteroid levels in the rat hypothalamus and entorhinal cortex. *Brain Research Bulletin, 68*(6), 419-424. http://dx.doi.org/10.1016/j.brainresbull.2005.09.015

Avital, A., & Richter-Levin, G. (2005). Exposure to juvenile stress exacerbates the behavioural consequences of exposure to stress in the adult rat. *Int.J.Neuropsychopharmacol., 8*(2), 163-173. http://dx.doi.org/10.1017/s1461145704004808

Ayensu, W. K., Pucilowski, O., Mason, G. A., Overstreet, D. H., Rezvani, A. H., & Janowsky, D. S. (1995). Effects of chronic mild stress on serum complement activity, saccharin preference, and corticosterone levels in Flinders lines of rats. *Physiol Behav., 57*(1), 165-169. doi:0031-9384(94)00204-I

Azpiroz, A., Fano, E., Garmendia, L., Arregi, A., Cacho, R., Beitia, G. et al. (1999). Effects of chronic mild stress (CMS) and imipramine administration, on spleen mononuclear cell proliferative response, serum corticosterone level and brain norepinephrine content in male mice. *Psychoneuroendocrinology, 24*(3), 345-361. doi:S0306-4530(98)00084-5

Bamberger, C. M., Schulte, H. M., & Chrousos, G. P. (1996). Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. *Endocr.Rev., 17*(3), 245-261. doi:10.1210/edrv-17-3-245

Barnett, S. A. (1958). Physiological effects of social stress in wild rats. I. The adrenal cortex. *J Psychosom.Res, 3*(1), 1-11. doi:0022-3999(58)90012-6

Barnett, S. A., Eaton, J. C., & McCallum, H. M. (1960). Physiological effects of "social stress" in wild rats--II. Liver glycogen and blood glucose. *J Psychosom.Res, 4*, 251-260. http://dx.doi.org/10.1016/0022-3999(60)90001-5

Bartolomucci, A., Cabassi, A., Govoni, P., Ceresini, G., Cero, C., Berra, D. et al. (2009). Metabolic consequences and vulnerability to diet-induced obesity in male mice under chronic social stress. *PLoS.One., 4*(1), e4331. doi:10.1371/journal.pone.0004331

Bartolomucci, A., Carola, V., Pascucci, T., Puglisi-Allegra, S., Cabib, S., Lesch, K. P. et al. (2010). Increased vulnerability to psychosocial stress in heterozygous serotonin transporter knockout mice. *Dis.Model.Mech., 3*(7-8), 459-470. doi:;10.1242/dmm.004614

Bartolomucci, A., Palanza, P., Gaspani, L., Limiroli, E., Panerai, A. E., Ceresini, G. et al. (2001). Social status in mice: behavioral, endocrine and immune changes are context dependent. *Physiol Behav., 73*(3), 401-410. doi:S0031-9384(01)00453-X

Bartolomucci, A., Palanza, P., Sacerdote, P., Panerai, A. E., Sgoifo, A., Dantzer, R. et al. (2005). Social factors and individual vulnerability to chronic stress exposure. *Neuroscience & Biobehavioral Reviews, 29*(1), 67-81. doi: 10.1016/j.neubiorev.2004.06.009

Bartolomucci, A., Pederzani, T., Sacerdote, P., Panerai, A. E., Parmigiani, S., & Palanza, P. (2004). Behavioral and physiological characterization of male mice under chronic psychosocial stress. *Psychoneuroendocrinology, 29*(7), 899-910. doi:10.1016/j.psyneuen.2003.08.003

Bastard, J. P., Maachi, M., Lagathu, C., Kim, M. J., Caron, M., Vidal, H. et al. (2006). Recent advances in the relationship between obesity, inflammation, and insulin resistance. *European Cytokine Network, 17*(1), 4-12. http://www.jle.com/en/revues/ecn/sommaire.phtml

Becker, C., Zeau, B., Rivat, C., Blugeot, A., Hamon, M., & Benoliel, J. J. (2008). Repeated social defeat-induced depression-like behavioral and biological alterations in rats: involvement of cholecystokinin. *Molecular Psychiatry, 13*(12), 1079-1092. doi:;10.1038/sj.mp.4002097

Bell, M. E., Bhargava, A., Soriano, L., Laugero, K., Akana, S. F., & Dallman, M. F. (2002). Sucrose intake and corticosterone interact with cold to modulate ingestive behaviour, energy balance, autonomic outflow and neuroendocrine responses during chronic stress. *Journal of Neuroendocrinology, 14*(4), 330-342. doi: 10.1046/j.1365-2826.2002.00784.x

Bell, M. E., Bhatnagar, S., Liang, J., Soriano, L., Nagy, T. R., & Dallman, M. F. (2000). Voluntary sucrose ingestion, like corticosterone replacement, prevents the metabolic

deficits of adrenalectomy. *Journal of Neuroendocrinology, 12*(5), 461-470. http://dx.doi.org/10.1046/j.1365-2826.2000.00488.x

Benelli, A., Filaferro, M., Bertolini, A., & Genedani, S. (1999). Influence of S-adenosyl-L-methionine on chronic mild stress-induced anhedonia in castrated rats. *Br.J.Pharmacol., 127*(3), 645-654. doi:10.1038/sj.bjp.0702589

Berton, O., Aguerre, S., Sarrieau, A., Mormede, P., & Chaouloff, F. (1998). Differential effects of social stress on central serotonergic activity and emotional reactivity in Lewis and spontaneously hypertensive rats. *Neuroscience, 82*(1), 147-159. doi:S0306452297002820

Berton, O., Durand, M., Aguerre, S., Mormede, P., & Chaouloff, F. (1999). Behavioral, neuroendocrine and serotonergic consequences of single social defeat and repeated fluoxetine pretreatment in the Lewis rat strain. *Neuroscience, 92*(1), 327-341. doi:S0306-4522(98)00742-8

Berton, O., McClung, C. A., DiLeone, R. J., Krishnan, V., Renthal, W., Russo, S. J. et al. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science, 311*(5762), 864-868. doi: 10.1126/science.1120972

Bertrand, E., Smadja, C., Mauborgne, A., Roques, B. P., & Dauge, V. (1997). Social interaction increases the extracellular levels of [Met]enkephalin in the nucleus accumbens of control but not of chronic mild stressed rats. *Neuroscience, 80*(1), 17-20. doi:S030645229700136X

Bhatnagar, S., Bell, M. E., Liang, J., Soriano, L., Nagy, T. R., & Dallman, M. F. (2000). Corticosterone facilitates saccharin intake in adrenalectomized rats: does corticosterone increase stimulus salience? *Journal of Neuroendocrinology, 12*(5), 453-460. http://dx.doi.org/10.1046/j.1365-2826.2000.00487.x

Bhatnagar, S., & Dallman, M. F. (1999). Neuroanatomical basis for facilitation of hypothalamic-pituitary-adrenal responses to a novel stressor after chronic stress. *Neuroscience, 84*, 1025-1039. http://dx.doi.org/10.1016/s0306-4522(97)00577-0

Bhatnagar, S., & Vining, C. (2003). Facilitation of hypothalamic-pituitary-adrenal responses to novel stress following repeated social stress using the resident/intruder paradigm. *Horm.Behav., 43*(1), 158-165. doi:S0018506X02000119

Bhatnagar, S., Vining, C., Iyer, V., & Kinni, V. (2006). Changes in hypothalamic-pituitary-adrenal function, body temperature, body weight and food intake with repeated social stress exposure in rats. *Journal of Neuroendocrinology, 18*(1), 13-24. doi: 10.1111/j.1365-2826.2005.01375.x

Bielajew, C., Konkle, A. T., Kentner, A. C., Baker, S. L., Stewart, A., Hutchins, A. A. et al. (2003). Strain and gender specific effects in the forced swim test: effects of previous stress exposure. *Stress., 6*(4), 269-280. doi:10.1080/1025389031000160282 9

Bielajew, C., Konkle, A. T. M., & Merali, Z. (2002). The effects of chronic mild stress on male Sprague-Dawley and Long Evans rats I. Biochemical and physiological analyses. *Behavioural Brain Research, 136*(2), 583-592. http://dx.doi.org/10.1016/s0166-4328(02)00222-x

Bingham, B., Gray, M., Sun, T., & Viau, V. (2011). Postnatal blockade of androgen receptors or aromatase impair the expression of stress hypothalamic-pituitary-adrenal axis habituation in adult male rats. *Psychoneuroendocrinology, 36*(2), 249-257. http://dx.doi.org/10.1016/j.psyneuen.2010.07.015

Bingham, B., McFadden, K., Zhang, X., Bhatnagar, S., Beck, S., & Valentino, R. (2011). Early adolescence as a critical window during which social stress distinctly alters behavior and brain norepinephrine activity. *Neuropharmacology, 36*, 896-909. http://dx.doi.org/10.1038/npp.2010.229

Blanchard, D. C., & Blanchard, R. J. (1990). Behavioral correlates of chronic dominance-subordination relationships of male rats in a seminatural situation. *Neuroscience & Biobehavioral Reviews, 14*(4), 455-462. http://dx.doi.org/10.1016/s0149-7634(05)80068-5

Blanchard, D. C., Sakai, R. R., McEwen, B., Weiss, S. M., & Blanchard, R. J. (1993). Subordination stress: Behavioral, brain, and neuroendocrine correlates. *Behav.Brain Res., 58*, 113-121. http://dx.doi.org/10.1016/0166-4328(93)90096-9

Blanchard, D. C., Spencer, R. L., Weiss, S. M., Blanchard, R. J., McEwen, B., & Sakai, R. R. (1995). Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. *Psychoneuroendocrinology, 20*(2), 117-134. doi:0306453094E0045B

Blanchard, R. J., & Blanchard, D. C. (1989). Antipredator defensive behaviors in a visible burrow system. *J.Comp Psychol., 103*(1), 70-82. http://dx.doi.org/10.1037/0735-7036.103.1.70

Blanchard, R. J., McKittrick, C. R., & Blanchard, D. C. (2001). Animal models of social stress: effects on behavior and brain neurochemical systems. *Physiology & Behavior, 73*, 261-271. http://dx.doi.org/10.1016/s0031-9384(01)00449-8

Bogdanova, O. V., Kanekar, S., D'Anci, K. E., & Renshaw, P. F. (2013). Factors influencing behavior in the forced swim test. *Physiol Behav., 118*, 227-239. doi: 10.1016/j.physbeh.2013.05.012

Bourke, C. H., Glasper, E. R., & Neigh, G. N. (2014). SSRI or CRF antagonism partially ameliorate depressive-like behavior after adolescent social defeat. *Behav.Brain Res., 270*, 295-299. doi: 10.1016/j.bbr.2014.05.035.

Bourke, C. H., & Neigh, G. N. (2011). Behavioral effects of chronic adolescent stress are sustained and sexually dimorphic. *Horm.Behav., 60*(1), 112-120. doi: 10.1016/j.yhbeh.2011.03.011

Brenes, J. C., Padilla, M., & Fornaguera, J. (2009). A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. *Behav.Brain Res., 197*(1), 125-137. doi: 10.1016/j.bbr.2008.08.014

Brenes, J. C., Rodriguez, O., & Fornaguera, J. (2008). Differential effect of environment enrichment and social isolation on depressive-like behavior, spontaneous activity and

serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum. *Pharmacology, Biochemistry and Behavior, 89*(1), 85-93. doi: 10.1016/j.pbb.2007.11.004 [doi].

Brotto, L. A., Gorzalka, B. B., & LaMarre, A. K. (2001). Melatonin protects against the effects of chronic stress on sexual behaviour in male rats. *Neuroreport, 12*(16), 3465-3469. http://dx.doi.org/10.1097/00001756-200111160-00018

Brydges, N. M., Hall, L., Nicolson, R., Holmes, M. C., & Hall, J. (2012). The effects of juvenile stress on anxiety, cognitive bias and decision making in adulthood: a rat model. *PLoS.One., 7*(10), e48143. doi:10.1371/journal.pone.0048143

Burke, A. R., & Miczek, K. A. (2014). Stress in adolescence and drugs of abuse in rodent models: role of dopamine, CRF, and HPA axis. *Psychopharmacology (Berl), 231*(8), 1557-1580. doi:10.1007/s00213-013-3369-1

Burke, A. R., Renner, K. J., Forster, G. L., & Watt, M. J. (2010). Adolescent social defeat alters neural, endocrine and behavioral responses to amphetamine in adult male rats. *Brain Research, 1352*, 147-156. doi: 10.1016/j.brainres.2010.06.062

Burke, A. R., Watt, M. J., & Forster, G. L. (2011). Adolescent social defeat increases adult amphetamine conditioned place preference and alters D2 dopamine receptor expression. *Neuroscience, 197*, 269-279. doi: 10.1016/j.neuroscience.2011.09.008

Buwalda, B., Blom, W. A., Koolhaas, J. M., & van Dijk, G. (2001). Behavioral and physiological responses to stress are affected by high-fat feeding in male rats. *Physiol Behav., 73*(3), 371-377. ttp://dx.doi.org/10.1016/s0031-9384(01)00493-0

Buwalda, B., De Boer, S. F., Schmidt, E. D., Felszeghy, K., Nyakas, C., Sgoifo, A. et al. (1999). Long-lasting deficient dexamethasone suppression of hypothalamic-pituitary-adrenocortical activation following peripheral CRF challenge in socially defeated rats. *Journal of Neuroendocrinology, 11*(7), 513-520. http://dx.doi.org/10.1046/j.1365-2826.1999.00350.x

Buwalda, B., Geerdink, M., Vidal, J., & Koolhaas, J. M. (2011). Social behavior and social stress in adolescence: a focus on animal models. *Neuroscience & Biobehavioral Reviews, 35*(8), 1713-1721. http://dx.doi.org/10.1016/j.neubiorev.2010.10.004

Buwalda, B., Stubbendorff, C., Zickert, N., & Koolhaas, J. M. (2013). Adolescent social stress does not necessarily lead to a compromised adaptive capacity during adulthood: a study on the consequences of social stress in rats. *Neuroscience*. http://dx.doi.org/10.1016/j.neuroscience.2012.12.050

Buynitsky, T., & Mostofsky, D. I. (2009). Restraint stress in biobehavioral research: Recent developments. *Neuroscience & Biobehavioral Reviews, 33*(7), 1089-1098. doi: 10.1016/j.neubiorev.2009.05.004

Byrne, D. G., Davenport, S. C., & Mazanov, J. (2007). Profiles of adolescent stress: the development of the adolescent stress questionnaire (ASQ). *J Adolesc., 30*(3), 393-416. doi: 10.1016/j.adolescence.2006.04.004

Calle, E. E., & Kaaks, R. (2004). Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat.Rev.Cancer, 4*(8), 579-591. http://dx.doi.org/10.1038/nrc1408

Calle, E. E., & Thun, M. J. (2004). Obesity and cancer. *Oncogene, 23*(38), 6365-6378. http://dx.doi.org/10.1136/bmj.39384.472072.80

Campos, A. C., Fogaca, M. V., Aguiar, D. C., & Guimaraes, F. S. (2013). Animal models of anxiety disorders and stress. *Rev.Bras.Psiquiatr., 35 Suppl 2*, S101-S111. doi:10.1590/1516-4446-2013-1139

Carlin, J., Hill-Smith, T. E., Lucki, I., & Reyes, T. M. (2013). Reversal of dopamine system dysfunction in response to high-fat diet. *Obesity.(Silver.Spring), 21*(12), 2513-2521. doi:10.1002/oby.20374

Carobrez, A. P., & Bertoglio, L. J. (2005). Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. *Neuroscience & Biobehavioral Reviews, 29*(8), 1193-1205. http://dx.doi.org/10.1016/j.neubiorev.2005.04.017

Cerf, M. E. (2007). High fat diet modulation of glucose sensing in the beta-cell. *Med.Sci.Monit., 13*(1), RA12-RA17. http://www.medscimonit.com/

Chang, A., Butler, S., Sliwoski, J., Valentino, R., Canning, D., & Zderic, S. (2009). Social stress in mice induces voiding dysfunction and bladder wall remodeling. *Am.J.Physiol Renal Physiol, 297*(4), F1101-F1108. doi: 10.1152/ajprenal.90749.2008

Chappell, A. M., Carter, E., McCool, B. A., & Weiner, J. L. (2013). Adolescent rearing conditions influence the relationship between initial anxiety-like behavior and ethanol drinking in male Long Evans rats. *Alcohol Clin.Exp.Res., 37 Suppl 1*, E394-E403. doi:10.1111/j.1530-0277.2012.01926.x

Charbonneau, A. M., Mezulis, A. H., & Hyde, J. S. (2009). Stress and emotional reactivity as explanations for gender differences in adolescents' depressive symptoms. *J Youth.Adolesc., 38*(8), 1050-1058. doi:10.1007/s10964-009-9398-8

Checkley, S. (1996). The neuroendocrinology of depression and chronic stress. *Br.Med.Bull., 52*(3), 597-617. http://dx.doi.org/10.1046/j.0007-1331.2001.00727.x

Chen, L., Faas, G. C., Ferando, I., & Mody, I. (2015). Novel insights into the behavioral analysis of mice subjected to the forced-swim test. *Transl.Psychiatry, 5*, e551. doi: 10.1038/tp.2015.44

Christiansen, A. M., Dekloet, A. D., Ulrich-Lai, Y. M., & Herman, J. P. (2011). "Snacking" causes long term attenuation of HPA axis stress responses and enhancement of brain FosB/deltaFosB expression in rats. *Physiol Behav., 103*(1), 111-116. http://dx.doi.org/10.1016/j.physbeh.2011.01.015

Christiansen, A. M., Herman, J. P., & Ulrich-Lai, Y. M. (2011). Regulatory interactions of stress and reward on rat forebrain opioidergic and GABAergic circuitry. *Stress., 14*(2), 205-215. doi: 10.3109/10253890.2010.531331

Chuang, J. C., Perello, M., Sakata, I., Osborne-Lawrence, S., Savitt, J. M., Lutter, M. et al. (2011). Ghrelin mediates stress-induced food-reward behavior in mice. *J.Clin.Invest, 121*(7), 2684-2692. doi: 10.1172/JCI57660.

Coccurello, R., D'Amato, F. R., & Moles, A. (2009). Chronic social stress, hedonism and vulnerability to obesity: lessons from rodents. *Neuroscience & Biobehavioral Reviews, 33*(4), 537-550. doi: 10.1016/j.neubiorev.2008.05.018.

Cohen, S., & Janicki-Deverts, D. (2012). Who's Stressed? Distributions of Psychological Stress in the United States in Probability Samples from 1983, 2006, and 2009. *Journal of Applied Social Psychology, 42*(6), 1320-1334. doi: 10.1111/j.1559-1816.2012.00900.x

Compas, B. E., Connor-Smith, J. K., Saltzman, H., Thomsen, A. H., & Wadsworth, M. E. (2001). Coping with stress during childhood and adolescence: problems, progress, and potential in theory and research. *Psychol.Bull., 127*(1), 87-127. doi: 10.1037//0033-2909.127.1.87.

Compas, B. E., Hinden, B. R., & Gerhardt, C. A. (1995). Adolescent development: pathways and processes of risk and resilience. *Annual Review of Psychology, 46*, 265-293. doi: 10.1146/annurev.ps.46.020195.001405

Conrad, C. D., LeDoux, J. E., Magarinos, A. M., & McEwen, B. S. (1999). Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. *Behav.Neurosci., 113*(5), 902-913. doi: 10.1037/0735-7044.113.5.902.

Coppens, C. M., Siripornmongcolchai, T., Wibrand, K., Alme, M. N., Buwalda, B., De Boer, S. F. et al. (2011). Social Defeat during Adolescence and Adulthood Differentially Induce BDNF-Regulated Immediate Early Genes. *Front Behav.Neurosci., 5*, 72. doi:10.3389/fnbeh.2011.00072

Correa, M., Arizzi, M. N., Betz, A., Mingote, S., & Salamone, J. D. (2003). Open filed locomotor effects in rats after intraventricular injections of ethanol and the ethnaol metabolites acetaldehyde and acetate. *Br.Res.Bull., 62*, 197-202. doi: 10.1016/j.brainresbull.2003.09.013

Covington, H. E., III, Kikusui, T., Goodhue, J., Nikulina, E. M., Hammer, R. P., Jr., & Miczek, K. A. (2005). Brief social defeat stress: long lasting effects on cocaine taking during a binge and zif268 mRNA expression in the amygdala and prefrontal cortex. *Neuropsychopharmacology, 30*(2), 310-321. doi: 10.1038/sj.npp.1300587

Cruz, F. C., DeLucia, R., & Planeta, C. S. (2008). Effects of chronic stress on nicotine-induced locomotor activity and corticosterone release in adult and adolescent rats. *Addict.Biol., 13*(1), 63-69. doi: 10.1111/j.1369-1600.2007.00080.x

Cruz, F. C., Marin, M. T., Leao, R. M., & Planeta, C. S. (2012). Stress-induced cross-sensitization to amphetamine is related to changes in the dopaminergic system. *J.Neural Transm., 119*(4), 415-424. doi:10.1007/s00702-011-0720-8

Cruz, M. L., Shaibi, G. Q., Weigensberg, M. J., Spruijt-Metz, D., Ball, G. D., & Goran, M. I. (2005). Pediatric obesity and insulin resistance: chronic disease risk and implications for

treatment and prevention beyond body weight modification. *Annual Review of Nutrition, 25*, 435-468. doi: 10.1146/annurev.nutr.25.050304.092625

Cryan, J. F., Markou, A., & Lucki, I. (2002). Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol.Sci, 23*(5), 238-245. doi:S0165-6147(02)02017-5

Cryan, J. F., Valentino, R. J., & Lucki, I. (2005). Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neuroscience & Biobehavioral Reviews, 29*(4-5), 547-569. doi: 10.1016/j.neubiorev.2005.03.008

Csikszentmihalyi, M., Larson, R., & Prescott, S. (1977). The ecology of adolescent activity and experience. *J.Youth.Adolesc., 6*(3), 281-294. doi:10.1007/BF02138940

D'Aquila, P., Monleon, S., Borsini, F., Brain, P., & Willner, P. (1997). Anti-anhedonic actions of the novel serotonergic agent flibanserin, a potential rapidly-acting antidepressant. *European Journal of Pharmacology, 340*(2-3), 121-132. doi:S0014-2999(97)01412-X

D'Aquila, P. S., Brain, P., & Willner, P. (1994). Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression. *Physiol Behav., 56*(5), 861-867. doi:0031-9384(94)90316-6

Dagher, A. (2009). The neurobiology of appetite: hunger as addiction. *Int.J.Obes.(Lond), 33 Suppl 2*, S30-S33. doi: 10.1038/ijo.2009.69.

Dallman, M. F. (2007). Modulation of the stress response: how we cope with excess glucocorticoids. *Experimental Neurology, 206*, 179-182. doi: 10.1016/j.expneurol.2007.06.002.

Dallman, M. F. (2010). Stress-induced obesity and the emotional nervous system. *Trends Endocrinol.Metab, 21*(3), 159-165. doi: 10.1016/j.tem.2009.10.004.

Dallman, M. F., Akana, S. F., Laugero, K. D., Gomez, F., Manalo, S., Bell, M. E. et al. (2003a). A spoonful of sugar: feedback signals of energy stores and corticosterone regulate responses to chronic stress. *Physiol Behav., 79*(1), 3-12. doi:S0031938403001008

Dallman, M. F., Levin, N., Cascio, C. S., Akana, S. F., Jacobson, L., & Kuhn, R. W. (1989). Pharmacological evidence that the inhibition of diurnal adrenocorticotropin secretion by corticosteroids is mediated via type I corticosterone-preferring receptors. *Endocrinology, 124*(6), 2844-2850. doi:10.1210/endo-124-6-2844

Dallman, M. F., Pecoraro, N., Akana, S. F., la Fleur, S. E., Gomez, F., Houshyar, H. et al. (2003b). Chronic stress and obesity: a new view of "comfort food". *Proc.Natl.Acad.Sci.U.S.A, 100*(20), 11696-11701. doi:10.1073/pnas.1934666100

Dallman, M. F., Pecoraro, N. C., & la Fleur, S. E. (2005). Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain Behav.Immun., 19*(4), 275-280. doi: 10.1016/j.bbi.2004.11.004

Dallman, M. F., Pecoraro, N. C., la Fleur, S. E., Warne, J. P., Ginsberg, A. B., Akana, S. F. et al. (2006). Glucocorticoids, chronic stress, and obesity. *Prog.Brain Res, 153*, 75-105. doi: 10.1073/pnas.1934666100

de Ferranti, S., & Mozaffarian, D. (2008). The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. *Clin.Chem., 54*(6), 945-955. doi: 10.1373/clinchem.2007.100156

De Goeij, D. C., Dijkstra, H., & Tilders, F. J. (1992). Chronic psychosocial stress enhances vasopressin, but not corticotropin-releasing factor, in the external zone of the median eminence of male rats: relationship to subordinate status. *Endocrinology, 131*(2), 847-853. http://dx.doi.org/10.1210/endo.131.2.1322285

de Kloet, E. R., Rots, N. Y., & Cools, A. R. (1996). Brain-Corticosteroid Hormone Dialogue: Slow and Persistent. *Cellular and Molecular Neurobiology 16*(3), 345-356. doi: 10.1007/BF02088100

De, V. J., & Schreiber, R. (1997). The chronic mild stress depression model: future developments from a drug discovery perspective. *Psychopharmacology (Berl), 134*(4), 349-350. doi: 10.1007/s002130050464

DeFronzo, R. A., & Ferrannini, E. (1991). Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care, 14*(3), 173-194. doi: **10.2337/diacare.14.3.173**

Demerath, E. W., Reed, D., Rogers, N., Sun, S. S., Lee, M., Choh, A. C. et al. (2008). Visceral adiposity and its anatomical distribution as predictors of the metabolic syndrome and cardiometabolic risk factor levels. *American Journal of Clinical Nutrition, 88*(5), 1263-1271. doi:88/5/1263

Despres, J. P. (2007). Cardiovascular disease under the influence of excess visceral fat. *Crit Pathw.Cardiol., 6*(2), 51-59. doi:10.1097/HPC.0b013e318057d4c9

Dess, N. K. (1992). Divergent responses to saccharin vs. sucrose availability after stress in rats. *Physiol Behav., 52*(1), 115-125. doi: 10.1016/0031-9384(92)90440-D

Detke, M. J., & Lucki, I. (1996). Detection of serotonergic and noradrenergic antidepressants in the rat forced swimming test: the effects of water depth. *Behav.Brain Res., 73*(1-2), 43-46. http://www.journals.elsevier.com/behavioural-brain-research/

Deuster, P. A., Singh, A., Hofmann, A., Moses, F. M., & Chrousos, G. C. (1992). Hormonal responses to ingesting water or a carbohydrate beverage during a 2 h run. *Medicine and Science in Sports and Exercise, 24*(1), 72-79. https://www.acsm.org/public-information/acsm-journals/medicine-science-in-sports-exercise

Di, C. G., Loddo, P., & Tanda, G. (1999). Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. *Biological Psychiatry, 46*(12), 1624-1633. doi:S0006-3223(99)00236-X

Di, C. G., & Tanda, G. (1997). Blunting of reactivity of dopamine transmission to palatable food: a biochemical marker of anhedonia in the CMS model? *Psychopharmacology (Berl), 134*(4), 351-353. doi: 10.1007/s002130050465

Di, S., Malcher-Lopes, R., Halmos, K. C., & Tasker, J. G. (2003). Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. *Journal of Neuroscience, 23*(12), 4850-4857. doi:23/12/4850

Dijkstra, H., Tilders, F. J., Hiehle, M. A., & Smelik, P. G. (1992). Hormonal reactions to fighting in rat colonies: prolactin rises during defence, not during offence. *Physiol Behav., 51*(5), 961-968. doi: 10.1016/0031-9384(92)90078-G

Diorio, D., Viau, V., & Meaney, M. J. (1993). The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *Journal of Neuroscience, 13*, 3839-3847. http://www.jneurosci.org/

Dong, C., Sanchez, L. E., & Price, R. A. (2004). Relationship of obesity to depression: a family-based study. *Int.J.Obes.Relat Metab Disord., 28*(6), 790-795. doi:10.1038/sj.ijo.0802626

Doremus, T. L., Varlinskaya, E. I., & Spear, L. P. (2004). Age-related differences in elevated plus maze behavior between adolescent and adult rats. *Ann.N.Y.Acad.Sci., 1021*, 427-430. doi: 1021/1/427

Dube, L., LeBel, J. L., & Lu, J. (2005). Affect asymmetry and comfort food consumption. *Physiol Behav., 86*(4), 559-567. http://dx.doi.org/10.1016/j.physbeh.2005.08.023

Ducottet, C., & Belzung, C. (2004). Behaviour in the elevated plus-maze predicts coping after subchronic mild stress in mice. *Physiol Behav., 81*(3), 417-426. doi:10.1016/j.physbeh.2004.01.013

Ducottet, C., Griebel, G., & Belzung, C. (2003). Effects of the selective nonpeptide corticotropin-releasing factor receptor 1 antagonist antalarmin in the chronic mild stress model of depression in mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 27*(4), 625-631. doi: 10.1016/S0278-5846(03)00051-4

Duman, R. S. (2002). Pathophysiology of depression: the concept of synaptic plasticity. *Eur.Psychiatry, 17 Suppl 3*, 306-310. doi:S0924933802006545

Duncko, R., Brtko, J., Kvetnansky, R., & Jezova, D. (2001). Altered function of peripheral organ systems in rats exposed to chronic mild stress model of depression. *Cell Mol.Neurobiol., 21*(4), 403-411. http://link.springer.com/journal/10571

Duncko, R., Kiss, A., Skultetyova, I., Rusnak, M., & Jezova, D. (2001). Corticotropin-releasing hormone mRNA levels in response to chronic mild stress rise in male but not in female rats while tyrosine hydroxylase mRNA levels decrease in both sexes. *Psychoneuroendocrinology, 26*(1), 77-89. http://dx.doi.org/10.1016/s0306-4530(00)00040-8

Dunn, J. D., & Whitener, J. (1986). Plasma corticosterone responses to electrical stimulation of the amygdaloid complex: cytoarchitectural specificity. *Neuroendocrinology, 42*(3), 211-217. http://dx.doi.org/10.1159/000124442

Eilam, D. (2003). Open-field behavior withstands drastic changes in arena size. *Behav.Brain Res., 142*(1-2), 53-62. doi:S0166432802003820

Einon, D. F., & Morgan, M. J. (1977). A critical period for social isolation in the rat. *Dev.Psychobiol., 10*(2), 123-132. doi:10.1002/dev.420100205

Einon, D. F., Morgan, M. J., & Kibbler, C. C. (1978). Brief periods of socialization and later behavior in the rat. *Dev.Psychobiol., 11*(3), 213-225. doi:10.1002/dev.420110305

Ely, D., Caplea, A., Dunphy, G., & Smith, D. (1997). Physiological and neuroendocrine correlates of social position in normotensive and hypertensive rat colonies. *Acta Physiol Scand.Suppl, 640*, 92-95. http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1748-1716/issues

Ely, D. L., & Henry, J. P. (1978). Neuroendocrine response patterns in dominant and subordinate mice. *Horm.Behav., 10*(2), 156-169. doi:0018-506X(78)90005-3

Fachin, A., Silva, R. K., Noschang, C. G., Pettenuzzo, L., Bertinetti, L., Billodre, M. N. et al. (2008). Stress effects on rats chronically receiving a highly palatable diet are sex-specific. *Appetite, 51*(3), 592-598. http://dx.doi.org/10.1016/j.appet.2008.04.016

Fekete, E. M., Zhao, Y., Li, C., Sabino, V., Vale, W. W., & Zorrilla, E. P. (2009). Social defeat stress activates medial amygdala cells that express type 2 corticotropin-releasing factor receptor mRNA. *Neuroscience, 162*(1), 5-13. doi: 10.1016/j.neuroscience.2009.03.078

Ferdman, N., Murmu, R. P., Bock, J., Braun, K., & Leshem, M. (2007). Weaning age, social isolation, and gender, interact to determine adult explorative and social behavior, and

dendritic and spine morphology in prefrontal cortex of rats. *Behav.Brain Res., 180*(2), 174-182. doi: 10.1016/j.bbr.2007.03.011

Ferre, P., Fernandez-Teruel, A., Escorihuela, R. M., Driscoll, P., Corda, M. G., Giorgi, O. et al. (1995). Behavior of the Roman/Verh high- and low-avoidance rat lines in anxiety tests: relationship with defecation and self-grooming. *Physiology & Behavior, 58*(6), 1209-1213. http://dx.doi.org/10.1016/0031-9384(95)02068-3

File, S. E., & Seth, P. (2003). A review of 25 years of the social interaction test. *European Journal of Pharmacology, 463*(1-3), 35-53. http://dx.doi.org/10.1016/s0014-2999(03)01273-1

Fone, K. C., & Porkess, M. V. (2008). Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. *Neuroscience & Biobehavioral Reviews, 32*(6), 1087-1102. doi: 10.1016/j.neubiorev.2008.03.003

Fone, K. C. F., & Dixon, D. M. (1991). Acute and chronic effects of intrathecal galanin on behavioural and biochemical markers of spinal motor function in adult rats. *Brain Research, 544*, 118-125. http://dx.doi.org/10.1016/0006-8993(91)90892-y

Foster, M. T., Solomon, M. B., Huhman, K. L., & Bartness, T. J. (2006). Social defeat increases food intake, body mass, and adiposity in Syrian hamsters. *Am.J.Physiol Regul.Integr.Comp Physiol, 290*(5), R1284-R1293. doi: 10.1152/ajpregu.00437.2005

Foster, M. T., Warne, J. P., Ginsberg, A. B., Horneman, H. F., Pecoraro, N. C., Akana, S. F. et al. (2009). Palatable foods, stress, and energy stores sculpt corticotropin-releasing factor, adrenocorticotropin, and corticosterone concentrations after restraint. *Endocrinology, 150*(5), 2325-2333. http://dx.doi.org/10.1210/jcem.94.2.9990

Fox, C. S., Massaro, J. M., Hoffmann, U., Pou, K. M., Maurovich-Horvat, P., Liu, C. Y. et al. (2007). Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation, 116*(1), 39-48. doi: 10.1161/CIRCULATIONAHA.106.675355

Freedman, D. S., Dietz, W. H., Srinivasan, S. R., & Berenson, G. S. (1999). The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study. *Pediatrics, 103*(6 Pt 1), 1175-1182. http://dx.doi.org/10.1542/peds.103.6.1175

Friedman, J. M. (2003). A war on obesity, not the obese. *Science, 299*(5608), 856-858. http://dx.doi.org/10.1126/science.1079856

Froger, N., Palazzo, E., Boni, C., Hanoun, N., Saurini, F., Joubert, C. et al. (2004). Neurochemical and behavioral alterations in glucocorticoid receptor-impaired transgenic mice after chronic mild stress. *Journal of Neuroscience, 24*(11), 2787-2796. doi:10.1523/JNEUROSCI.4132-03.2004 [doi];24/11/2787

Furay, A. R., Bruestle, A. E., & Herman, J. P. (2008). The role of the forebrain glucocorticoid receptor in acute and chronic stress. *Endocrinology, 149*(11), 5482-5490. doi: 10.1210/en.2008-0642

Gamaro, G. D., Manoli, L. P., Torres, I. L., Silveira, R., & Dalmaz, C. (2003). Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures. *Neurochemistry International, 42*(2), 107-114. doi:S0197018602000803

Gamaro, G. D., Prediger, M. E., Lopes, J. B., & Dalmaz, C. (2003). Interaction between estradiol replacement and chronic stress on feeding behavior and on serum leptin. *Pharmacology, Biochemistry and Behavior, 76*(2), 327-333. doi:S0091305703002259

Gambarana, C., Ghiglieri, O., Tolu, P., De Montis, M. G., Giachetti, D., Bombardelli, E. et al. (1999a). Efficacy of an Hypericum perforatum (St. John's wort) extract in preventing and reverting a condition of escape deficit in rats. *Neuropsychopharmacology, 21*(2), 247-257. doi: 10.1016/S0893-133X(99)00027-5

Gambarana, C., Masi, F., Tagliamonte, A., Scheggi, S., Ghiglieri, O., & De Montis, M. G. (1999b). A chronic stress that impairs reactivity in rats also decreases dopaminergic transmission in the nucleus accumbens: a microdialysis study. *J.Neurochem., 72*(5), 2039-2046. doi: 10.1046/j.1471-4159.1999.0722039.x

Gambarana, C., Scheggi, S., Tagliamonte, A., Tolu, P., & De Montis, M. G. (2001). Animal models for the study of antidepressant activity. *Brain Research.Brain Research Protocols, 7*(1), 11-20. doi:S1385-299X(00)00056-8

Gameiro, G. H., Gameiro, P. H., Andrade, A. S., Pereira, L. F., Arthuri, M. T., Marcondes, F. K. et al. (2006). Nociception- and anxiety-like behavior in rats submitted to different periods of restraint stress. *Physiol Behav., 87*(4), 643-649. doi: 10.1016/j.physbeh.2005.12.007

Garcia-Marquez, C., & Armario, A. (1987). Chronic stress depresses exploratory activity and behavioral performance in the forced swimming test without altering ACTH response to a novel acute stressor. *Physiol Behav., 40*(1), 33-38. doi:0031-9384(87)90182-X

Gardner, K. L., Thrivikraman, K. V., Lightman, S. L., Plotsky, P. M., & Lowry, C. A. (2005). Early life experience alters behavior during social defeat: focus on serotonergic systems. *Neuroscience, 136*(1), 181-191. doi: 10.1016/j.neuroscience.2005.07.042

Garg, N., Thakur, S., Alex, M. C., & Adamo, M. L. (2011). High fat diet induced insulin resistance and glucose intolerance are gender-specific in IGF-1R heterozygous mice. *Biochem.Biophys.Res Commun.*. http://dx.doi.org/10.1016/j.bbrc.2011.08.123

Gibson, E. L. (2006). Emotional influences on food choice: sensory, physiological and psychological pathways. *Physiol Behav., 89*(1), 53-61. http://dx.doi.org/10.1016/j.physbeh.2006.01.024

Gil, M., Nguyen, N. T., McDonald, M., & Albers, H. E. (2013). Social reward: interactions with social status, social communication, aggression, and associated neural activation in the ventral tegmental area. *European Journal of Neuroscience, 38*(2), 2308-2318. doi:10.1111/ejn.12216

Giriko, C. A., Andreoli, C. A., Mennitti, L. V., Hosoume, L. F., Souto, T. S., Silva, A. V. et al. (2013). Delayed physical and neurobehavioral development and increased aggressive and depression-like behaviors in the rat offspring of dams fed a high-fat diet. *Int.J.Dev.Neurosci., 31*(8), 731-739. doi: 10.1016/j.ijdevneu.2013.09.001

Glavin, G. B., Pare, W. P., Sandbak, T., Bakke, H. K., & Murison, R. (1994). Restraint stress in biomedical research: an update. *Neuroscience & Biobehavioral Reviews, 18*(2), 223-249. doi:0149-7634(94)90027-2

Gomez, F., Houshyar, H., & Dallman, M. F. (2002). Marked regulatory shifts in gonadal, adrenal, and metabolic system responses to repeated restraint stress occur within a 3-week period in pubertal male rats. *Endocrinology, 143*(8), 2852-2862. doi:10.1210/endo.143.8.8929

Gordon-Larsen, P., Adair, L. S., Nelson, M. C., & Popkin, B. M. (2004). Five-year obesity incidence in the transition period between adolescence and adulthood: the National Longitudinal Study of Adolescent Health. *American Journal of Clinical Nutrition, 80*(3), 569-575. http://ajcn.nutrition.org/

Graham, Y. P., Heim, C., Goodman, S. H., Miller, A. H., & Nemeroff, C. B. (1999). The effects of neonatal stress on brain development: implications for psychopathology. *Development and Psychopathology, 11*(3), 545-565. http://dx.doi.org/10.1017/s0954579499002205

Greaves-Lord, K., Ferdinand, R. F., Oldehinkel, A. J., Sondeijker, F. E., Ormel, J., & Verhulst, F. C. (2007). Higher cortisol awakening response in young adolescents with persistent anxiety problems. *Acta Psychiatr.Scand., 116*(2), 137-144. http://dx.doi.org/10.1111/j.1600-0447.2007.01001.x

Green, M. R., Barnes, B., & McCormick, C. M. (2013). Social instability stress in adolescence increases anxiety and reduces social interactions in adulthood in male Long-Evans rats. *Dev.Psychobiol., 55*(8), 849-859. doi:10.1002/dev.21077

Green, M. R., & McCormick, C. M. (2013). Effects of stressors in adolescence on learning and memory in rodent models. *Horm.Behav., 64*(2), 364-379. doi: 10.1016/j.yhbeh.2012.09.012

Griebel, G., Rodgers, R. J., Perrault, G., & Sanger, D. J. (1997). Risk assessment behaviour: evaluation of utility in the study of 5-HT-related drugs in the rat elevated plus-maze test. *Pharmacology, Biochemistry & Behavior, 57*(4), 817-827. http://dx.doi.org/10.1016/s0091-3057(96)00402-9

Griebel, G., Simiand, J., Serradeil-Le, G. C., Wagnon, J., Pascal, M., Scatton, B. et al. (2002a). Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. *Proc.Natl.Acad.Sci.U.S.A, 99*(9), 6370-6375. doi:10.1073/pnas.092012099

Griebel, G., Simiand, J., Steinberg, R., Jung, M., Gully, D., Roger, P. et al. (2002b). 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylp henyl)ethyl]5-methyl-N-(2-propynyl)-1, 3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor(1) receptor antagonist. II. Characterization in rodent models of stress-related disorders. *Journal of Pharmacology & Experimental Therapeutics, 301*(1), 333-345. http://jpet.aspetjournals.org/

Griebel, G., Stemmelin, J., & Scatton, B. (2005). Effects of the cannabinoid CB1 receptor antagonist rimonabant in models of emotional reactivity in rodents. *Biological Psychiatry, 57*(3), 261-267. doi: 10.1016/j.biopsych.2004.10.032

Griffiths, J., Shanks, N., & Anisman, H. (1992). Strain dependent alterations in food consumption following acute stressor exposure. *Pharmacology, Biochemistry and Behavior, 42*, 219-227. http://dx.doi.org/10.1016/0091-3057(92)90519-l

Grippo, A. J., Beltz, T. G., & Johnson, A. K. (2003). Behavioral and cardiovascular changes in the chronic mild stress model of depression. *Physiol Behav., 78*(4-5), 703-710. http://dx.doi.org/10.1016/s0031-9384(03)00050-7

Grippo, A. J., Francis, J., Beltz, T. G., Felder, R. B., & Johnson, A. K. (2005). Neuroendocrine and cytokine profile of chronic mild stress-induced anhedonia. *Physiol Behav., 84*(5), 697-706. doi: 10.1016/j.physbeh.2005.02.011

Grippo, A. J., Moffitt, J. A., & Johnson, A. K. (2002). Cardiovascular alterations and autonomic imbalance in an experimental model of depression. *Am.J.Physiol Regul.Integr.Comp Physiol, 282*(5), R1333-R1341. doi:10.1152/ajpregu.00614.2001

Grippo, A. J., Na, E. S., Johnson, R. F., Beltz, T. G., & Johnson, A. K. (2004). Sucrose ingestion elicits reduced Fos expression in the nucleus accumbens of anhedonic rats. *Brain Research, 1019*(1-2), 259-264. doi:10.1016/j.brainres.2004.05.019

Grippo, A. J., Sullivan, N. R., Damjanoska, K. J., Crane, J. W., Carrasco, G. A., Shi, J. et al. (2005). Chronic mild stress induces behavioral and physiological changes, and may alter serotonin 1A receptor function, in male and cycling female rats. *Psychopharmacology (Berl), 179*(4), 769-780. doi:10.1007/s00213-004-2103-4

Gronli, J., Murison, R., Bjorvatn, B., Sorensen, E., Portas, C. M., Ursin, R. et al. (2004). Chronic mild stress affects sucrose intake and sleep in rats. *Behavioural Brain Research, 150*(1-2), 139-147. http://dx.doi.org/10.1016/s0166-4328(03)00252-3

Gronli, J., Murison, R., Fiske, E., Bjorvatn, B., Sorensen, E., Portas, C. M. et al. (2005). Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. *Physiol Behav., 84*(4), 571-577. doi: 10.1016/j.physbeh.2005.02.007

Gust, D. A., Gordon, T. P., Brodie, A. R., & McClure, H. M. (1996). Effect of companions in modulating stress associated with new group formation in juvenile rhesus macaques. *Physiol Behav., 59*(4-5), 941-945. doi:0031-9384(95)02164-7

Hall, F. S. (1998). Social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioral consequences. *Crit Rev.Neurobiol., 12*(1-2), 129-162. http://dx.doi.org/10.1615/critrevneurobiol.v12.i1-2.50

Hall, F. S., Huang, S., Fong, G. F., & Pert, A. (1998). The effects of social isolation on the forced swim test in Fawn hooded and Wistar rats. *Journal of Neuroscience Methods, 79*(1), 47-51. http://dx.doi.org/10.1016/s0165-0270(97)00155-6

Hall, F. S., Huang, S., Fong, G. W., Pert, A., & Linnoila, M. (1998). Effects of isolation-rearing on locomotion, anxiety and responses to ethanol in Fawn Hooded and Wistar rats. *Psychopharmacology (Berl), 139*(3), 203-209. http://dx.doi.org/10.1007/s002130050705

Hall, F. S., Huang, S., Fong, G. W., Sundstrom, J. M., & Pert, A. (2000). Differential basis of strain and rearing effects on open-field behavior in Fawn Hooded and Wistar rats. *Physiol Behav., 71*(5), 525-532. http://dx.doi.org/10.1016/s0031-9384(00)00372-3

Hall, F. S., Sundstrom, J. M., Lerner, J., & Pert, A. (2001). Enhanced corticosterone release after a modified forced swim test in Fawn hooded rats is independent of rearing experience. *Pharmacology, Biochemistry and Behavior, 69*(3-4), 629-634. http://dx.doi.org/10.1016/s0091-3057(01)00556-1

Haller, J., Fuchs, E., Halasz, J., & Makara, G. B. (1999). Defeat is a major stressor in males while social instability is stressful mainly in females: towards the development of a social stress model in female rats. *Brain Res Bull., 50*(1), 33-39. http://dx.doi.org/10.1016/s0361-9230(99)00087-8

Harris, R. B., Mitchell, T. D., Simpson, J., Redmann, S. M., Jr., Youngblood, B. D., & Ryan, D. H. (2002). Weight loss in rats exposed to repeated acute restraint stress is independent of energy or leptin status. *Am.J.Physiol Regul.Integr.Comp Physiol, 282*(1), R77-R88. http://ajpregu.physiology.org/

Harris, R. B., Zhou, J., Youngblood, B. D., Smagin, G. N., & Ryan, D. H. (1997). Failure to change exploration or saccharin preference in rats exposed to chronic mild stress. *Physiol Behav., 63*(1), 91-100. http://dx.doi.org/10.1016/s0031-9384(97)00425-3

Harro, J., Haidkind, R., Harro, M., Modiri, A. R., Gillberg, P. G., Pahkla, R. et al. (1999). Chronic mild unpredictable stress after noradrenergic denervation: attenuation of behavioural and biochemical effects of DSP-4 treatment. *European*

Neuropsychopharmacology, 10(1), 5-16. http://dx.doi.org/10.1016/s0924-977x(99)00043-7

Hatch, A., Wiberg, G. S., Balazs, T., & Grice, H. C. (1963). Long-term Isolation Stress in Rats. *Science, 142*, 507. http://dx.doi.org/10.1126/science.142.3591.507

Hatch, A. M., Wiberg, G. S., Zawidzka, Z., Cann, M., Airth, J. M., & Grice, H. C. (1965). Isolation syndrome in the rat. *Toxicology and Applied Pharmacology, 7*(5), 737-745. http://dx.doi.org/10.1016/0041-008x(65)90132-8

Hatcher, J. P., Bell, D. J., Reed, T. J., & Hagan, J. J. (1997). Chronic mild stress-induced reductions in saccharin intake depend upon feeding status. *J.Psychopharmacol., 11*(4), 331-338. http://dx.doi.org/10.1177/026988119701100408

Hauger, R. L., Lorang, M., Irwin, M., & Aguilera, G. (1990). CRF receptor regulation and sensitization of ACTH responses to acute ether stress during chronic intermittent immobilization stress. *Brain Research, 532*, 34-40. http://dx.doi.org/10.1016/0006-8993(90)91738-3

Hauger, R. L., Millan, M. A., Lorang, M., Harwood, J. P., & Aguilera, G. (1988). Corticotropin-releasing factor receptors and pituitary adrenal responses during immobilization stress. *Endocrinology, 123*(1), 396-405. http://dx.doi.org/10.1210/endo-123-1-396

Hayashida, S., Oka, T., Mera, T., & Tsuji, S. (2010). Repeated social defeat stress induces chronic hyperthermia in rats. *Physiol Behav., 101*(1), 124-131. doi: 10.1016/j.physbeh.2010.04.027

Heidbreder, C. A., Weiss, I. C., Domeney, A. M., Pryce, C., Homberg, J., Hedou, G. et al. (2000). Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome. *Neuroscience, 100*(4), 749-768. http://dx.doi.org/10.1016/s0306-4522(00)00336-5

Heim, C., & Nemeroff, C. B. (1999). The impact of early adverse experiences on brain systems involved in the pathophysiology of anxiety and affective disorders. *Biological Psychiatry, 46*(11), 1509-1522. http://dx.doi.org/10.1016/s0006-3223(99)00224-3

Heinrichs, S. C., Menzaghi, F., Pich, E. M., Baldwin, H. A., Rassnick, S., Britton, K. T. et al. (1994). Anti-stress action of a corticotropin-releasing factor antagonist on behavioral reactivity to stressors of varying type and intensity. *Neuropsychopharmacology, 11*, 179-186. http://dx.doi.org/10.1038/sj.npp.1380104

Heinrichs, S. C., Pich, E. M., Miczek, K. A., Britton, K. T., & Koob, G. F. (1992). Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. *Brain Research, 581*(2), 190-197. http://dx.doi.org/10.1016/0006-8993(92)90708-h

Hellemans, K. G., Benge, L. C., & Olmstead, M. C. (2004). Adolescent enrichment partially reverses the social isolation syndrome. *Brain Res.Dev.Brain Res., 150*(2), 103-115. doi:10.1016/j.devbrainres.2004.03.003

Herman, J. P., Adams, D., & Prewitt, C. (1995). Regulatory changes in neuroendocrine stress-integrative circuitry produced by a variable stress paradigm. *Neuroendocrinology, 61*(2), 180-190. http://dx.doi.org/10.1159/000126839

Herman, J. P., & Cullinan, W. E. (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci., 20*, 78-84. http://dx.doi.org/10.1016/s0166-2236(96)10069-2

Herman, J. P., Figueiredo, H., Mueller, N. K., & Ulrich-Lai, Y. (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Frontiers in Neuroendocrinology, 24*(3), 151-180. http://dx.doi.org/10.1016/j.yfrne.2003.07.001

Hill, J. O., & Peters, J. C. (1998). Environmental contributions to the obesity epidemic. *Science, 280*(5368), 1371-1374. http://dx.doi.org/10.1126/science.280.5368.1371

Hill, M. N., Hellemans, K. G., Verma, P., Gorzalka, B. B., & Weinberg, J. (2012). Neurobiology of chronic mild stress: parallels to major depression. *Neuroscience & Biobehavioral Reviews, 36*(9), 2085-2117. doi: 10.1016/j.neubiorev.2012.07.001

Hill, R. A., Kiss Von, S. S., Ratnayake, U., Klug, M., Binder, M. D., Hannan, A. J. et al. (2014). Long-term effects of combined neonatal and adolescent stress on brain-derived neurotrophic factor and dopamine receptor expression in the rat forebrain. *Biochimica et Biophysica Acta, 1842*(11), 2126-2135. doi: 10.1016/j.bbadis.2014.08.009

Hills, A. P., Andersen, L. B., & Byrne, N. M. (2011). Physical activity and obesity in children. *British Journal of Sports Medicine, 45*(11), 866-870. doi: 10.1136/bjsports-2011-090199

Hollis, F., Wang, H., Dietz, D., Gunjan, A., & Kabbaj, M. (2010). The effects of repeated social defeat on long-term depressive-like behavior and short-term histone modifications in the

hippocampus in male Sprague-Dawley rats. *Psychopharmacology (Berl), 211*(1), 69-77. doi:10.1007/s00213-010-1869-9

Holmes, A., Le Guisquet, A. M., Vogel, E., Millstein, R. A., Leman, S., & Belzung, C. (2005). Early life genetic, epigenetic and environmental factors shaping emotionality in rodents. *Neuroscience & Biobehavioral Reviews, 29*(8), 1335-1346. doi: 10.1016/j.neubiorev.2005.04.012

Holson, R. R., Scallet, A. C., Ali, S. F., & Turner, B. B. (1991). "Isolation stress" revisited: isolation-rearing effects depend on animal care methods. *Physiol Behav., 49*(6), 1107-1118. http://dx.doi.org/10.1016/0031-9384(91)90338-o

Hong, S., Flashner, B., Chiu, M., ver, H. E., Luz, S., & Bhatnagar, S. (2012). Social isolation in adolescence alters behaviors in the forced swim and sucrose preference tests in female but not in male rats. *Physiol Behav., 105*(2), 269-275. http://dx.doi.org/10.1016/j.physbeh.2011.08.036

Horovitz, O., Tsoory, M. M., Hall, J., Jacobson-Pick, S., & Richter-Levin, G. (2012). Post-weaning to pre-pubertal ('juvenile') stress: a model of induced predisposition to stress-related disorders. *Neuroendocrinology, 95*(1), 56-64. doi: 10.1159/000331393

Horovitz, O., Tsoory, M. M., Yovell, Y., & Richter-Levin, G. (2014). A rat model of pre-puberty (Juvenile) stress-induced predisposition to stress-related disorders: sex similarities and sex differences in effects and symptoms. *World J.Biol.Psychiatry, 15*(1), 36-48. doi:10.3109/15622975.2012.745604

Ikemoto, S., & Panksepp, J. (1992). The effects of early social isolation on the motivation for social play in juvenile rats. *Dev.Psychobiol., 25*(4), 261-274. doi:10.1002/dev.420250404

Ilin, Y., & Richter-Levin, G. (2009). Enriched environment experience overcomes learning deficits and depressive-like behavior induced by juvenile stress. *PLoS.One., 4*(1), e4329. doi:10.1371/journal.pone.0004329

Iniguez, S. D., Riggs, L. M., Nieto, S. J., Dayrit, G., Zamora, N. N., Shawhan, K. L. et al. (2014). Social defeat stress induces a depression-like phenotype in adolescent male c57BL/6 mice. *Stress., 17*(3), 247-255. doi:10.3109/10253890.2014.910650

Inoue, K., Kiriike, N., Okuno, M., Ito, H., Fujisaki, Y., Matsui, T. et al. (1993). Scheduled feeding caused activation of dopamine metabolism in the striatum of rats. *Physiol.Behav., 53*, 177-181. http://dx.doi.org/10.1016/0031-9384(93)90028-e

Inoue, T., Koyama, T., Muraki, A., & Yamashita, I. (1993). Effects of single and repeated immobilization stress on corticotropin-releasing factor concentrations in discrete rat brain regions. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 17*(1), 161-170. http://dx.doi.org/10.1016/0278-5846(93)90040-y

International Obesity Task Force. (2010). Obesity: The Global Epidemic. *International Obesity Task Force Website*. Retrieved from http://www.iaso.org/iotf/obesity/obesitytheglobalepidemic/

Isgor, C., Kabbaj, M., Akil, H., & Watson, S. J. (2004). Delayed effects of chronic variable stress during peripubertal-juvenile period on hippocampal morphology and on cognitive and stress axis functions in rats. *Hippocampus, 14*(5), 636-648. doi:10.1002/hipo.10207

Isovich, E., Engelmann, M., Landgraf, R., & Fuchs, E. (2001). Social isolation after a single defeat reduces striatal dopamine transporter binding in rats. *European Journal of Neuroscience, 13*(6), 1254-1256. http://dx.doi.org/10.1046/j.0953-816x.2001.01492.x

Itoh, S., Takashima, A., Itoh, T., & Morimoto, T. (1994). Open-field behavior of rats following intracerebroventricular administration of neuromedin B, neuromedin C, and related amphibian peptides. *JPN.J.Physiol., 44*, 271-281. http://dx.doi.org/10.2170/jjphysiol.44.271

Jacobson, L., & Sapolsky, R. (1991). The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocr.Rev., 12*(2), 118-134. doi:10.1210/edrv-12-2-118

Jacobson-Pick, S., Audet, M. C., Nathoo, N., & Anisman, H. (2011). Stressor experiences during the juvenile period increase stressor responsivity in adulthood: transmission of stressor experiences. *Behav.Brain Res, 216*(1), 365-374. http://dx.doi.org/10.1016/j.bbr.2010.08.016

Jacobson-Pick, S., Elkobi, A., Vander, S., Rosenblum, K., & Richter-Levin, G. (2008). Juvenile stress-induced alteration of maturation of the GABAA receptor alpha subunit in the rat. *Int.J.Neuropsychopharmacol., 11*(7), 891-903. http://dx.doi.org/10.1017/s1461145708008559

Jacobson-Pick, S., & Richter-Levin, G. (2010). Differential impact of juvenile stress and corticosterone in juvenility and in adulthood, in male and female rats. *Behav.Brain Res, 214*(2), 268-276. http://dx.doi.org/10.1016/j.bbr.2010.05.036

Jacobson-Pick, S., & Richter-Levin, G. (2012). Short- and long-term effects of juvenile stressor exposure on the expression of GABAA receptor subunits in rats. *Stress., 15*(4), 416-424. doi:10.3109/10253890.2011.634036

Jankord, R., Solomon, M. B., Albertz, J., Flak, J. N., Zhang, R., & Herman, J. P. (2011). Stress vulnerability during adolescent development in rats. *Endocrinology, 152*(2), 629-638. doi: 10.1210/en.2010-0658

Jankowska, E., Pucilowski, O., & Kostowski, W. (1991). Chronic oral treatment with diltiazem or verapamil decreases isolation-induced activity impairment in elevated plus maze. *Behav.Brain Res., 43*(2), 155-158. http://dx.doi.org/10.1016/s0166-4328(05)80065-8

Jee, S. H., Kim, H. J., & Lee, J. (2005). Obesity, insulin resistance and cancer risk. *Yonsei Med.J., 46*(4), 449-455. http://dx.doi.org/10.3349/ymj.2005.46.4.449

Juvonen, J., Graham, S., & Schuster, M. A. (2003). Bullying among young adolescents: the strong, the weak, and the troubled. *Pediatrics, 112*(6 Pt 1), 1231-1237. http://dx.doi.org/10.1542/peds.112.6.1231

Kallen, V. L., Tulen, J. H., Utens, E. M., Treffers, P. D., De Jong, F. H., & Ferdinand, R. F. (2008). Associations between HPA axis functioning and level of anxiety in children and

adolescents with an anxiety disorder. *Depress.Anxiety., 25*(2), 131-141. http://dx.doi.org/10.1002/da.20287

Kalueff, A. V., & Tuohimaa, P. (2004). Experimental modeling of anxiety and depression. *Acta Neurobiol.Exp.(Wars.), 64*(4), 439-448. http://www.ane.pl/index.php

Kampe, J., Tschop, M. H., Hollis, J. H., & Oldfield, B. J. (2009). An anatomic basis for the communication of hypothalamic, cortical and mesolimbic circuitry in the regulation of energy balance. *Eur J Neurosci., 30*(3), 415-430. http://dx.doi.org/10.1111/j.1460-9568.2009.06818.x

Kandel, E. R., Schwartz, J. H., Jessell, T. M., Seigelbaum, S. A., & Hudspeth, A. J. (2013). *Principles of Neural Science* (5th ed.). New York: McGraw Hill.

Kang, J. E., Cirrito, J. R., Dong, H., Csernansky, J. G., & Holtzman, D. M. (2007). Acute stress increases interstitial fluid amyloid-beta via corticotropin-releasing factor and neuronal activity. *Proc.Natl.Acad.Sci.U.S.A, 104*(25), 10673-10678. doi: 10.1073/pnas.0700148104

Kant, G. J., & Bauman, R. A. (1993). Effects of chronic stress and time of day on preference for sucrose. *Physiol Behav., 54*(3), 499-502. http://dx.doi.org/10.1016/0031-9384(93)90242-8

Karatsoreos, I. N., & McEwen, B. S. (2013). Annual Research Review: The neurobiology and physiology of resilience and adaptation across the life course. *Journal of Child*

Psychology and Psychiatry and Allied Disciplines, 54(4), 337-347. doi:10.1111/jcpp.12054

Kasckow, J. W., Lupien, S. J., Behan, D. P., Welge, J., & Hauger, R. J. (2001). Circulating human corticotropin-releasing factor-binding protein levels following cortisol infusions. *Life Sciences, 69*(2), 133-142. http://dx.doi.org/10.1016/s0024-3205(01)01108-0

Katz, R. J. (1982). Animal model of depression: pharmacological sensitivity of a hedonic deficit. *Pharmacology, Biochemistry and Behavior, 16*(6), 965-968. http://dx.doi.org/10.1016/0091-3057(82)90053-3

Keeney, A., Jessop, D. S., Harbuz, M. S., Marsden, C. A., Hogg, S., & Blackburn-Munro, R. E. (2006). Differential effects of acute and chronic social defeat stress on hypothalamic-pituitary-adrenal axis function and hippocampal serotonin release in mice. *Journal of Neuroendocrinology, 18*(5), 330-338. doi:10.1111/j.1365-2826.2006.01422.x

Keller-Wood, M. E., & Dallman, M. F. (1984). Corticosteroid inhibition of ACTH secretion. *Endocrine Reviews, 5*(1), 1-24. http://dx.doi.org/10.1210/edrv-5-1-1

Kelley, A. E., Baldo, B. A., Pratt, W. E., & Will, M. J. (2005). Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiol Behav., 86*(5), 773-795. http://dx.doi.org/10.1016/j.physbeh.2005.08.066

Kelly, T., Yang, W., Chen, C. S., Reynolds, K., & He, J. (2008). Global burden of obesity in 2005 and projections to 2030. *Int.J.Obes.(Lond), 32*(9), 1431-1437. doi: 10.1038/ijo.2008.102

Kendler, K. S., Karkowski, L. M., & Prescott, C. A. (1999). Causal relationship between stressful life events and the onset of major depression. *American Journal of Psychiatry, 156*(6), 837-841. http://dx.doi.org/10.1176/ajp.156.6.837

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry, 62*(6), 593-602. doi: 10.1001/archpsyc.62.6.593

Khan, S., & Liberzon, I. (2004). Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. *Psychopharmacology (Berl), 172*(2), 225-229. doi:10.1007/s00213-003-1634-4

Kim, C. K., Yu, W., Edin, G., Ellis, L., Osborn, J. A., & Weinberg, J. (1999). Chronic intermittent stress does not differentially alter brain corticosteroid receptor densities in rats prenatally exposed to ethanol. *Psychoneuroendocrinology, 24*(6), 585-611. http://dx.doi.org/10.1016/s0306-4530(99)00015-3

Kim, S. H., Han, J., Seog, D. H., Chung, J. Y., Kim, N., Hong, P. Y. et al. (2005). Antidepressant effect of Chaihu-Shugan-San extract and its constituents in rat models of depression. *Life Sci., 76*(11), 1297-1306. doi: 10.1016/j.lfs.2004.10.022

Kim, Y. S., & Leventhal, B. (2008). Bullying and Suicide. A review. *International Journal of Adolescent Medicine and Health, 20*, 133-154. http://dx.doi.org/10.1515/ijamh.2008.20.2.133

Kioukia, N., Bekris, S., Antoniou, K., Papadopoulou-Daifoti, Z., & Christofidis, I. (2000). Effects of chronic mild stress (CMS) on thyroid hormone function in two rat strains. *Psychoneuroendocrinology, 25*(3), 247-257. http://dx.doi.org/10.1016/s0306-4530(99)00051-7

Kiyokawa, Y., Kikusui, T., Takeuchi, Y., & Mori, Y. (2004). Partner's stress status influences social buffering effects in rats. *Behav.Neurosci., 118*(4), 798-804. doi:10.1037/0735-7044.118.4.798

Knight, J. A. (2011). Diseases and disorders associated with excess body weight. *Ann.Clin.Lab Sci., 41*(2), 107-121. http://www.annclinlabsci.org/

Kompagne, H., Bardos, G., Szenasi, G., Gacsalyi, I., Harsing, L. G., & Levay, G. (2008). Chronic mild stress generates clear depressive but ambiguous anxiety-like behaviour in rats. *Behav.Brain Res., 193*(2), 311-314. doi:10.1016/j.bbr.2008.06.008

Konkle, A. T., Baker, S. L., Kentner, A. C., Barbagallo, L. S., Merali, Z., & Bielajew, C. (2003). Evaluation of the effects of chronic mild stressors on hedonic and physiological responses: sex and strain compared. *Brain Research, 992*(2), 227-238. http://dx.doi.org/10.1016/j.brainres.2003.08.047

Koolhaas, J. M., Coppens, C. M., De Boer, S. F., Buwalda, B., Meerlo, P., & Timmermans, P. J. (2013). The resident-intruder paradigm: a standardized test for aggression, violence and social stress. *J.Vis.Exp.,*(77), e4367. doi:10.3791/4367

Koolhaas, J. M., De Boer, S. F., De Rutter, A. J., Meerlo, P., & Sgoifo, A. (1997). Social stress in rats and mice. *Acta Physiol Scand.Suppl, 640*, 69-72. http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1748-1716

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van der Vegt, B. J., Van Reenen, C. G., Hopster, H. et al. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews, 23*(7), 925-935. http://dx.doi.org/10.1016/s0149-7634(99)00026-3

Koolhaas, J. M., Meerlo, P., De Boer, S. F., Strubbe, J. H., & Bohus, B. (1997). The temporal dynamics of the stress response. *Neuroscience & Biobehavioral Reviews, 21*(6), 775-782. http://dx.doi.org/10.1016/s0149-7634(96)00057-7

Kopp, C., Vogel, E., Rettori, M. C., Delagrange, P., & Misslin, R. (1999). The effects of melatonin on the behavioural disturbances induced by chronic mild stress in C3H/He mice. *Behavioural Pharmacology, 10*(1), 73-83. http://dx.doi.org/10.1097/00008877-199902000-00007

Korner, J., & Leibel, R. L. (2003). To eat or not to eat - how the gut talks to the brain. *New England Journal of Medicine, 349*(10), 926-928. http://dx.doi.org/10.1056/nejmp038114

Kovalenko, I. L., Galyamina, A. G., Smagin, D. A., Michurina, T. V., Kudryavtseva, N. N., & Enikolopov, G. (2014). Extended effect of chronic social defeat stress in childhood on behaviors in adulthood. *PLoS.One., 9*(3), e91762. doi:10.1371/journal.pone.0091762

Krahn, D. D., Gosnell, B. A., Grace, M., & Levine, A. S. (1986). CRF antagonist partially reverses CRF- and stress-induced effects on feeding. *Br.Res.Bull., 17*, 285-289. http://dx.doi.org/10.1016/0361-9230(86)90233-9

Kriketos, A. D., Greenfield, J. R., Peake, P. W., Furler, S. M., Denyer, G. S., Charlesworth, J. A. et al. (2004). Inflammation, insulin resistance, and adiposity: a study of first-degree relatives of type 2 diabetic subjects. *Diabetes Care, 27*(8), 2033-2040. http://dx.doi.org/10.2337/diacare.27.8.2033

Krishnan, V., Han, M. H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J. et al. (2007). Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell, 131*(2), 391-404. doi: 10.1016/j.cell.2007.09.018

Krolow, R., Noschang, C., Arcego, D. M., Huffell, A. P., Marcolin, M. L., Benitz, A. N. et al. (2013a). Sex-specific effects of isolation stress and consumption of palatable diet during the prepubertal period on metabolic parameters. *Metabolism, 62*(9), 1268-1278. doi: 10.1016/j.metabol.2013.04.009

Krolow, R., Noschang, C., Arcego, D. M., Pettenuzzo, L. F., Weis, S. N., Marcolin, M. L. et al. (2013b). Isolation stress exposure and consumption of palatable diet during the prepubertal period leads to cellular changes in the hippocampus. *Neurochemical Research, 38*(2), 262-272. doi:10.1007/s11064-012-0915-x

Kudryavtseva, N. N. (2003). Use of the "partition" test in behavioral and pharmacological experiments. *Neurosci.Behav.Physiol, 33*(5), 461-471. http://www.springer.com/biomed/neuroscience/journal/11055

Kumar, V., Bhat, Z. A., & Kumar, D. (2013). Animal models of anxiety: a comprehensive review. *J.Pharmacol.Toxicol.Methods, 68*(2), 175-183. doi: 10.1016/j.vascn.2013.05.003

Kuo, L. E., Kitlinska, J. B., Tilan, J. U., Li, L., Baker, S. B., Johnson, M. D. et al. (2007). Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nature Medicine, 13*(7), 803-811. doi: 10.1038/nm1611

Kyrou, I., Chrousos, G. P., & Tsigos, C. (2006). Stress, visceral obesity, and metabolic complications. *Ann N.Y.Acad Sci, 1083*, 77-110. http://dx.doi.org/10.1196/annals.1367.008

Kyrou, I., & Tsigos, C. (2009). Stress hormones: physiological stress and regulation of metabolism. *Curr Opin.Pharmacol., 9*(6), 787-793. doi: 10.1016/j.coph.2009.08.007

la Fleur, S. E., Houshyar, H., Roy, M., & Dallman, M. F. (2005). Choice of lard, but not total lard calories, damps adrenocorticotropin responses to restraint. *Endocrinology, 146*(5), 2193-2199. http://dx.doi.org/10.1210/en.2004-1603

la Fleur, S. E., Luijendijk, M. C., van der Zwaal, E. M., Brans, M. A., & Adan, R. A. (2014). The snacking rat as model of human obesity: effects of a free-choice high-fat high-sugar diet on meal patterns. *Int.J.Obes.(Lond), 38*(5), 643-649. doi: 10.1038/ijo.2013.159

Lakehayli, S., Said, N., Khachibi, M. E., Ouahli, M. E., Nadifi, S., Hakkou, F. et al. (2015). Long-term effects of prenatal stress and diazepam on D2 receptor expression in the nucleus accumbens of adult rats. *Neuroscience Letters, 594*, 133-136. doi: 10.1016/j.neulet.2015.03.065

Lapiz, M. D., Fulford, A., Muchimapura, S., Mason, R., Parker, T., & Marsden, C. A. (2003). Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. *Neurosci.Behav.Physiol, 33*(1), 13-29. http://www.springer.com/biomed/neuroscience/journal/11055

Laugero, K. D., Bell, M. E., Bhatnagar, S., Soriano, L., & Dallman, M. F. (2001). Sucrose ingestion normalizes central expression of corticotropin-releasing-factor messenger ribonucleic acid and energy balance in adrenalectomized rats: a glucocorticoid-metabolic-brain axis? *Endocrinology, 142*(7), 2796-2804. http://dx.doi.org/10.1210/en.142.7.2796

Laursen, B., & Collins, W. A. (1994). Interpersonal conflict during adolescence. *Psychol.Bull., 115*(2), 197-209. http://dx.doi.org/10.1037/0033-2909.115.2.197

LeDoux, J. E. (1995). Emotion: clues from the brain. *Annual Review of Psychology, 46*, 209-235. http://dx.doi.org/10.1146/annurev.psych.46.1.209

Lee, A. L., Ogle, W. O., & Sapolsky, R. M. (2002). Stress and depression: possible links to neuron death in the hippocampus. *Bipolar.Disord., 4*(2), 117-128. http://dx.doi.org/10.1034/j.1399-5618.2002.01144.x

Lee, J. H., Kim, J. Y., & Jahng, J. W. (2014). Highly Palatable Food during Adolescence Improves Anxiety-Like Behaviors and Hypothalamic-Pituitary-Adrenal Axis Dysfunction in Rats that Experienced Neonatal Maternal Separation. *Endocrinol.Metab (Seoul.), 29*(2), 169-178. doi:10.3803/EnM.2014.29.2.169

Lepsch, L. B., Gonzalo, L. A., Magro, F. J., DeLucia, R., Scavone, C., & Planeta, C. S. (2005). Exposure to chronic stress increases the locomotor response to cocaine and the basal levels of corticosterone in adolescent rats. *Addict.Biol., 10*(3), 251-256. doi: 10.1080/13556210500269366

Levin, B. E. (1996). Reduced paraventricular nucleus norepinephrine responsiveness in obesity-prone rats. *Am.J.Physiol, 270*(2 Pt 2), R456-R461. http://www.physiology.org/

Levine, A. S., & Billington, C. J. (1997). Why do we eat? A neural systems approach. *Annual Review of Nutrition, 17*, 597-619. http://dx.doi.org/10.1146/annurev.nutr.17.1.597

Li, Y., Zheng, X., Liang, J., & Peng, Y. (2010). Coexistence of Anhedonia and anxiety-independent increased novelty-seeking behavior in the chronic mild stress model of depression. *Behavioural Processes, 83*, 331-339. http://dx.doi.org/10.1016/j.beproc.2010.01.020

Llorente, R., Miguel-Blanco, C., Aisa, B., Lachize, S., Borcel, E., Meijer, O. C. et al. (2011). Long term sex-dependent psychoneuroendocrine effects of maternal deprivation and juvenile unpredictable stress in rats. *Journal of Neuroendocrinology, 23*(4), 329-344. doi:10.1111/j.1365-2826.2011.02109.x

Lodge, D. J., & Lawrence, A. J. (2003). The effect of isolation rearing on volitional ethanol consumption and central CCK/dopamine systems in Fawn-Hooded rats. *Behav.Brain Res., 141*(2), 113-122. http://dx.doi.org/10.1016/s0166-4328(02)00328-5 Lopez-Duran, N. L., Kovacs, M., & George, C. J. (2009). Hypothalamic-pituitary-adrenal axis

dysregulation in depressed children and adolescents: a meta-analysis. *Psychoneuroendocrinology, 34*(9), 1272-1283. 10.1016/j.psyneuen.2009.03.016

Lucas, L. R., Wang, C. J., McCall, T. J., & McEwen, B. S. (2007). Effects of immobilization stress on neurochemical markers in the motivational system of the male rat. *Brain Research, 1155*, 108-115. doi: 10.1016/j.brainres.2007.04.063

Lucki, I. (1997). The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behavioural Pharmacology, 8*(6-7), 523-532. http://dx.doi.org/10.1097/00008877-199711000-00010

Lukkes, J., Vuong, S., Scholl, J., Oliver, H., & Forster, G. (2009). Corticotropin-releasing factor receptor antagonism within the dorsal raphe nucleus reduces social anxiety-like behavior after early-life social isolation. *Journal of Neuroscience, 29*(32), 9955-9960. doi: 10.1523/JNEUROSCI.0854-09.2009

Lukkes, J. L., Mokin, M. V., Scholl, J. L., & Forster, G. L. (2009). Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. *Horm.Behav., 55*(1), 248-256. doi: 10.1016/j.yhbeh.2008.10.014

Lukkes, J. L., Summers, C. H., Scholl, J. L., Renner, K. J., & Forster, G. L. (2009). Early life social isolation alters corticotropin-releasing factor responses in adult rats. *Neuroscience, 158*(2), 845-855. doi: 10.1016/j.neuroscience.2008.10.036

Luo, L., & Tan, R. X. (2001). Fluoxetine inhibits dendrite atrophy of hippocampal neurons by decreasing nitric oxide synthase expression in rat depression model. *Acta Pharmacol.Sin., 22*(10), 865-870. http://www.chinaphar.com/

Luo, X. M., Yuan, S. N., Guan, X. T., Xie, X., Shao, F., & Wang, W. W. (2014). Juvenile stress affects anxiety-like behavior and limbic monoamines in adult rats. *Physiol Behav., 135*, 7-16. doi: 10.1016/j.physbeh.2014.05.035

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience, 10*, 434-445. http://dx.doi.org/10.1038/nrn2639

Lutter, M., & Nestler, E. J. (2009). Homeostatic and hedonic signals interact in the regulation of food intake. *J.Nutr., 139*(3), 629-632. http://dx.doi.org/10.3945/jn.108.097618

Lutter, M., Sakata, I., Osborne-Lawrence, S., Rovinsky, S. A., Anderson, J. G., Jung, S. et al. (2008). The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nature Neuroscience, 11*(7), 752-753. doi: 10.1038/nn.2139

Ma, X. M., Lightman, S. L., & Aguilera, G. (1999). Vasopressin and corticotropin-releasing hormone gene responses to novel stress in rats adapted to repeated restraint. *Endocrinology, 140*(8), 3623-3632. doi:10.1210/endo.140.8.6943

Machado, T. D., Dalle, M. R., Laureano, D. P., Portella, A. K., Werlang, I. C., Benetti, C. S. et al. (2013). Early life stress is associated with anxiety, increased stress responsivity and

preference for "comfort foods" in adult female rats. *Stress., 16*(5), 549-556. doi:10.3109/10253890.2013.816841

Macht, M. (2008). How emotions affect eating: a five-way model. *Appetite, 50*(1), 1-11. http://dx.doi.org/10.1016/j.appet.2007.07.002

Mackay, J. C., James, J. S., Cayer, C., Kent, P., Anisman, H., & Merali, Z. (2014). Protracted effects of juvenile stressor exposure are mitigated by access to palatable food. *PLoS.One., 9*(5), e96573. doi:10.1371/journal.pone.0096573

Maisonnette, S., Morato, S., & Brandao, M. L. (1993). Role of resocialization and of 5-HT1A receptor activation on the anxiogenic effects induced by isolation in the elevated plus-maze test. *Physiol Behav., 54*(4), 753-758. http://dx.doi.org/10.1016/0031-9384(93)90087-v

Maniam, J., & Morris, M. J. (2010a). Palatable cafeteria diet ameliorates anxiety and depression-like symptoms following an adverse early environment. *Psychoneuroendocrinology, 35*(5), 717-728. http://dx.doi.org/10.1016/j.psyneuen.2009.10.013

Maniam, J., & Morris, M. J. (2010b). Voluntary exercise and palatable high-fat diet both improve behavioural profile and stress responses in male rats exposed to early life stress: role of hippocampus. *Psychoneuroendocrinology, 35*(10), 1553-1564. doi: 10.1016/j.psyneuen.2010.05.012

Mao, Q. Q., Ip, S. P., Ko, K. M., Tsai, S. H., & Che, C. T. (2009). Peony glycosides produce antidepressant-like action in mice exposed to chronic unpredictable mild stress: effects on

hypothalamic-pituitary-adrenal function and brain-derived neurotrophic factor. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 33*(7), 1211-1216. doi: 10.1016/j.pnpbp.2009.07.002

Marcolin, M. L., Benitz, A. N., Arcego, D. M., Noschang, C., Krolow, R., & Dalmaz, C. (2012). Effects of early life interventions and palatable diet on anxiety and on oxidative stress in young rats. *Physiol Behav., 106*(4), 491-498. doi: 10.1016/j.physbeh.2012.03.025

Marissal-Arvy, N., Gaumont, A., Langlois, A., Dabertrand, F., Bouchecareilh, M., Tridon, C. et al. (2007). Strain differences in hypothalamic pituitary adrenocortical axis function and adipogenic effects of corticosterone in rats. *Journal of Endocrinology, 195*(3), 473-484. doi:;10.1677/JOE-07-0077

Markus, R., Panhuysen, G., Tuiten, A., & Koppeschaar, H. (2000). Effects of food on cortisol and mood in vulnerable subjects under controllable and uncontrollable stress. *Physiol Behav., 70*(3-4), 333-342. http://dx.doi.org/10.1016/s0031-9384(00)00265-1

Marona-Lewicka, D., & Nichols, D. E. (1997). The Effect of Selective Serotonin Releasing Agents in the Chronic Mild Stress Model of Depression in Rats. *Stress., 2*(2), 91-100. http://dx.doi.org/10.3109/10253899709014740

Marti, O., Gavalda, A., Gomez, F., & Armario, A. (1994). Direct evidence for chronic stress-induced facilitation of the adrenocorticotropin response to a novel acute stressor. *Neuroendocrinology, 60*(1), 1-7. http://dx.doi.org/10.1159/000126713

Marti, O., Gavalda, A., Jolin, T., & Armario, A. (1996). Acute stress attenuates but does not abolish circadian rhythmicity of serum thyrotrophin and growth hormone in the rat. *European Journal of Endocrinology / European Federation of Endocrine Societies, 135*(6), 703-708. http://dx.doi.org/10.1530/eje.0.1350703

Martinez, M., Phillips, P. J., & Herbert, J. (1998). Adaptation in patterns of c-fos expression in the brain associated with exposure to either single or repeated social stress in male rats. *European Journal of Neuroscience, 10*(1), 20-33. http://dx.doi.org/10.1046/j.1460-9568.1998.00011.x

Maslova, L. N., Bulygina, V. V., & Markel, A. L. (2002). Chronic stress during prepubertal development: immediate and long-lasting effects on arterial blood pressure and anxiety-related behavior. *Psychoneuroendocrinology, 27*(5), 549-561. http://dx.doi.org/10.1016/s0306-4530(01)00092-0

Mathews, I. Z., Mills, R. G., & McCormick, C. M. (2008). Chronic social stress in adolescence influenced both amphetamine conditioned place preference and locomotor sensitization. *Dev.Psychobiol., 50*(5), 451-459. http://dx.doi.org/10.1002/dev.20299

Mathews, I. Z., Wilton, A., Styles, A., & McCormick, C. M. (2008). Increased depressive behaviour in females and heightened corticosterone release in males to swim stress after adolescent social stress in rats. *Behav.Brain Res., 190*(1), 33-40. doi: 10.1016/j.bbr.2008.02.004

Matthews, K., Forbes, N., & Reid, I. C. (1995). Sucrose consumption as an hedonic measure following chronic unpredictable mild stress. *Physiol Behav., 57*(2), 241-248. http://dx.doi.org/10.1016/0031-9384(94)00286-e

McCormick, C. M., & Green, M. R. (2013). From the stressed adolescent to the anxious and depressed adult: investigations in rodent models. *Neuroscience, 249*, 242-257. doi: 10.1016/j.neuroscience.2012.08.063

McCormick, C. M., Green, M. R., Cameron, N. M., Nixon, F., Levy, M. J., & Clark, R. A. (2013). Deficits in male sexual behavior in adulthood after social instability stress in adolescence in rats. *Horm.Behav., 63*(1), 5-12. doi: 10.1016/j.yhbeh.2012.11.009

McCormick, C. M., Kehoe, P., Mallinson, K., Cecchi, L., & Frye, C. A. (2002). Neonatal isolation alters stress hormone and mesolimbic dopamine release in juvenile rats. *Pharmacology, Biochemistry and Behavior, 73*(1), 77-85. http://dx.doi.org/10.1016/s0091-3057(02)00758-x

McCormick, C. M., & Mathews, I. Z. (2007). HPA function in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors. *Pharmacology, Biochemistry and Behavior, 86*(2), 220-233. http://dx.doi.org/10.1016/j.pbb.2006.07.012

McCormick, C. M., & Mathews, I. Z. (2010). Adolescent development, hypothalamic-pituitary-adrenal function, and programming of adult learning and memory. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 34*(5), 756-765. http://dx.doi.org/10.1016/j.pnpbp.2009.09.019

McCormick, C. M., Mathews, I. Z., Thomas, C., & Waters, P. (2010). Investigations of HPA function and the enduring consequences of stressors in adolescence in animal models. *Brain Cogn, 72*(1), 73-85. doi: 10.1016/j.bandc.2009.06.003

McCormick, C. M., Merrick, A., Secen, J., & Helmreich, D. L. (2007). Social instability in adolescence alters the central and peripheral hypothalamic-pituitary-adrenal responses to a repeated homotypic stressor in male and female rats. *Journal of Neuroendocrinology, 19*(2), 116-126. doi: 10.1111/j.1365-2826.2006.01515.x

McCormick, C. M., Nixon, F., Thomas, C., Lowie, B., & Dyck, J. (2010). Hippocampal cell proliferation and spatial memory performance after social instability stress in adolescence in female rats. *Behav.Brain Res., 208*(1), 23-29. doi: 10.1016/j.bbr.2009.11.003

McCormick, C. M., Rioux, T., Fisher, R., Lang, K., MacLaury, K., & Teillon, S. M. (2001). Effects of neonatal corticosterone treatment on maze performance and HPA axis in juvenile rats. *Physiol Behav., 74*(3), 371-379. http://dx.doi.org/10.1016/s0031-9384(01)00574-1

McCormick, C. M., Robarts, D., Kopeikina, K., & Kelsey, J. E. (2005). Long-lasting, sex- and age-specific effects of social stressors on corticosterone responses to restraint and on locomotor responses to psychostimulants in rats. *Horm.Behav., 48*(1), 64-74. http://dx.doi.org/10.1016/j.yhbeh.2005.01.008

McCormick, C. M., Smith, C., & Mathews, I. Z. (2008). Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. *Beh Brain Res, 187*, 228-238. http://dx.doi.org/10.1016/j.bbr.2007.09.005

McCormick, C. M., Thomas, C. M., Sheridan, C. S., Nixon, F., Flynn, J. A., & Mathews, I. Z. (2012). Social instability stress in adolescent male rats alters hippocampal neurogenesis and produces deficits in spatial location memory in adulthood. *Hippocampus, 22*(6), 1300-1312. doi:10.1002/hipo.20966

McEwen, B. S. (2012). The ever-changing brain: cellular and molecular mechanisms for the effects of stressful experiences. *Dev.Neurobiol., 72*(6), 878-890. doi:10.1002/dneu.20968

McEwen, B. S., & Stellar, E. (1993). Stress and the individual. Mechanisms leading to disease. *Archives of Internal Medicine, 153*(18), 2093-2101. http://dx.doi.org/10.1001/archinte.1993.00410180039004

McGuire, M. T., Brammer, G. L., & Raleigh, M. J. (1986). Resting cortisol levels and the emergence of dominant status among male vervet monkeys. *Horm.Behav., 20*(1), 106-117. http://dx.doi.org/10.1016/0018-506x(86)90033-4

McIlwain, K. L., Merriweather, M. Y., Yuva-Paylor, L. A., & Paylor, R. (2001). The use of behavioral test batteries: effects of training history. *Physiol Behav., 73*(5), 705-717. http://dx.doi.org/10.1016/s0031-9384(01)00528-5

McKittrick, C. R., Blanchard, D. C., Blanchard, R. J., McEwen, B. S., & Sakai, R. R. (1995). Serotonin receptor binding in a colony model of chronic social stress. *Biological Psychiatry, 37*(6), 383-393. http://dx.doi.org/10.1016/0006-3223(94)00152-s

McKittrick, C. R., Magarinos, A. M., Blanchard, D. C., Blanchard, R. J., McEwen, B. S., & Sakai, R. R. (2000). Chronic social stress reduces dendritic arbors in CA3 of

hippocampus and decreases binding to serotonin transporter sites. *Synapse, 36*(2), 85-94. http://dx.doi.org/10.1002/(sici)1098-2396(200005)36:2<85::aid-syn1>3.0.co;2-y

McLaughlin, K. J., Gomez, J. L., Baran, S. E., & Conrad, C. D. (2007). The effects of chronic stress on hippocampal morphology and function: an evaluation of chronic restraint paradigms. *Brain Research, 1161*, 56-64. doi: 10.1016/j.brainres.2007.05.042

Meerlo, P., Overkamp, G. J., Benning, M. A., Koolhaas, J. M., & Van Den Hoofdakker, R. H. (1996). Long-term changes in open field behaviour following a single social defeat in rats can be reversed by sleep deprivation. *Physiol Behav., 60*(1), 115-119. http://dx.doi.org/10.1016/0031-9384(95)02271-6

Meerlo, P., Overkamp, G. J., Daan, S., Van Den Hoofdakker, R. H., & Koolhaas, J. M. (1996). Changes in Behaviour and Body Weight Following a Single or Double Social Defeat in Rats. *Stress., 1*(1), 21-32. http://dx.doi.org/10.3109/10253899609001093

Meerlo, P., Sgoifo, A., De Boer, S. F., & Koolhaas, J. M. (1999). Long-lasting consequences of a social conflict in rats: behavior during the interaction predicts subsequent changes in daily rhythms of heart rate, temperature, and activity. *Behav.Neurosci., 113*(6), 1283-1290. http://dx.doi.org/10.1037/0735-7044.113.6.1283

Mela, V., Llorente-Berzal, A., Diaz, F., Argente, J., Viveros, M. P., & Chowen, J. A. (2012). Maternal deprivation exacerbates the response to a high fat diet in a sexually dimorphic manner. *PLoS.One., 7*(11), e48915. doi:10.1371/journal.pone.0048915

Mendonca, F. H., & Guimaraes, F. S. (1998). Intra-hippocampal administration of cycloheximide attenuates the restraint-induced exploratory deficit of an elevated plus maze. *Behav.Brain Res., 91*(1-2), 207-211. doi:S0166-4328(97)00129-0

Mendoza, S. P., Coe, C. L., Lowe, E. L., & Levine, S. (1978). The physiological response to group formation in adult male squirrel monkeys. *Psychoneuroendocrinology, 3*(3-4), 221-229. http://dx.doi.org/10.1016/0306-4530(78)90012-4

Merali, Z., Hayley, S., Kent, P., McIntosh, J., Bedard, T., & Anisman, H. (2009). Impact of repeated stressor exposure on the release of corticotropin-releasing factor, arginine-vasopressin and bombesin-like peptides at the anterior pituitary. *Beh Brain Res, 198*, 105-112. http://dx.doi.org/10.1016/j.bbr.2008.10.025

Meye, F. J., & Adan, R. A. (2014). Feelings about food: the ventral tegmental area in food reward and emotional eating. *Trends Pharmacol.Sci, 35*(1), 31-40. doi: 10.1016/j.tips.2013.11.003

Michel, C., Levin, B. E., & Dunn-Meynell, A. A. (2003). Stress facilitates body weight gain in genetically predisposed rats on medium-fat diet. *Am.J.Physiol Regul.Integr.Comp Physiol, 285*(4), R791-R799. doi:10.1152/ajpregu.00072.2003

Miczek, K. A. (1979). A new test for aggression in rats without aversive stimulation: differential effects of d-amphetamine and cocaine. *Psychopharmacology (Berl), 60*, 253-259. http://dx.doi.org/10.1007/bf00426664

Miczek, K. A., Covington, H. E., III, Nikulina, E. M., Jr., & Hammer, R. P. (2004). Aggression and defeat: persistent effects on cocaine self-administration and gene expression in peptidergic and aminergic mesocorticolimbic circuits. *Neuroscience & Biobehavioral Reviews, 27*(8), 787-802. doi:10.1016/j.neubiorev.2003.11.005

Mineur, Y. S., Belzung, C., & Crusio, W. E. (2006). Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. *Behav.Brain Res., 175*(1), 43-50. doi:.1016/j.bbr.2006.07.029

Mineur, Y. S., Prasol, D. J., Belzung, C., & Crusio, W. E. (2003). Agonistic behavior and unpredictable chronic mild stress in mice. *Behavior Genetics, 33*(5), 513-519. http://dx.doi.org/10.1016/j.bbr.2006.07.029

Mizoguchi, K., Ishige, A., Aburada, M., & Tabira, T. (2003). Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. *Neuroscience, 119*(3), 887-897. http://dx.doi.org/10.1016/s0306-4522(03)00105-2

Moksnes, U. K., Byrne, D. G., Mazanov, J., & Espnes, G. A. (2010). Adolescent stress: evaluation of the factor structure of the Adolescent Stress Questionnaire (ASQ-N). *Scand.J Psychol, 51*(3), 203-209. doi: 10.1111/j.1467-9450.2009.00803.x

Molcho, M., Craig, W., Due, P., Pickett, W., Harel-Fisch, Y., & Overpeck, M. (2009). Cross-national time trends in bullying behaviour 1994-2006: findings from Europe and North America. *Int.J.Public Health, 54 Suppl 2*, 225-234. doi:10.1007/s00038-009-5414-8

Moles, A., Bartolomucci, A., Garbugino, L., Conti, R., Caprioli, A., Coccurello, R. et al. (2006). Psychosocial stress affects energy balance in mice: modulation by social status. *Psychoneuroendocrinology, 31*(5), 623-633. doi: 10.1016/j.psyneuen.2006.01.004

Molet, J., Maras, P. M., Avishai-Eliner, S., & Baram, T. Z. (2014). Naturalistic rodent models of chronic early-life stress. *Dev.Psychobiol.*. doi:10.1002/dev.21230

Molina, V. A., Heyser, C. J., & Spear, L. P. (1994). Chronic variable stress or chronic morphine facilitates immobility in a forced swim test: reversal by naloxone. *Psychopharmacology (Berl), 114*(3), 433-440. http://dx.doi.org/10.1007/bf02249333

Moreau, J. L., Bourson, A., Jenck, F., Martin, J. R., & Mortas, P. (1994). Curative effects of the atypical antidepressant mianserin in the chronic mild stress-induced anhedonia model of depression. *J.Psychiatry Neurosci., 19*(1), 51-56. http://dx.doi.org/10.1097/00008877-199511000-00003

Moreau, J. L., Jenck, F., Martin, J. R., Mortas, P., & Haefely, W. (1993). Effects of moclobemide, a new generation reversible Mao-A inhibitor, in a novel animal model of depression. *Pharmacopsychiatry, 26*(1), 30-33. doi:10.1055/s-2007-1014338

Moreau, J. L., Scherschlicht, R., Jenck, F., & Martin, J. R. (1995). Chronic mild stress-induced anhedonia model of depression: Sleep abnormalities and curative effects of electroshock treatment. *Behavioural Pharmacology, 6*, 682-687. http://dx.doi.org/10.1097/00008877-199511000-00003

Murray, K. M., Byrne, D. G., & Rieger, E. (2011). Investigating adolescent stress and body image. *J.Adolesc., 34*(2), 269-278. http://dx.doi.org/10.1016/j.adolescence.2010.05.004

Murua, V. S., Gomez, R. A., Andrea, M. E., & Molina, V. A. (1991). Shuttle-box deficits induced by chronic variable stress: reversal by imipramine administration. *Pharmacology, Biochemistry and Behavior, 38*(1), 125-130. http://dx.doi.org/10.1016/0091-3057(91)90599-w

Muscat, R., Papp, M., & Willner, P. (1992). Reversal of stress-induced anhedonia by the atypical antidepressants, fluoxetine and maprotiline. *Psychopharmacology, 109*, 433-438. http://dx.doi.org/10.1007/bf02247719

Muscat, R., Towell, A., & Willner, P. (1988). Changes in dopamine autoreceptor sensitivity in an animal model of depression. *Psychopharmacology (Berl), 94*(4), 545-550. http://dx.doi.org/10.1007/bf00212853

Muscat, R., & Willner, P. (1992). Suppression of sucrose drinking by chronic mild unpredictable stress: A methodological analysis. *Neuroscience & Biobehavioral Reviews, 16*, 507-517. http://dx.doi.org/10.1016/s0149-7634(05)80192-7

Negron-Oyarzo, I., Perez, M. A., Terreros, G., Munoz, P., & Dagnino-Subiabre, A. (2014). Effects of chronic stress in adolescence on learned fear, anxiety, and synaptic transmission in the rat prelimbic cortex. *Behav.Brain Res., 259*, 342-353. doi: 10.1016/j.bbr.2013.11.001

Nestler, E. J., Gould, E., Manji, H., Buncan, M., Duman, R. S., Greshenfeld, H. K. et al. (2002). Preclinical models: status of basic research in depression. *Biological Psychiatry, 52*(6), 503-528. http://dx.doi.org/10.1016/s0006-3223(02)01405-1

Nielsen, C. K., Arnt, J., & Sanchez, C. (2000). Intracranial self-stimulation and sucrose intake differ as hedonic measures following chronic mild stress: interstrain and interindividual differences. *Behav.Brain Res., 107*(1-2), 21-33. http://dx.doi.org/10.1016/s0166-4328(99)00110-2

Niesink, R. J., & Van Ree, J. M. (1982a). Antidepressant drugs normalize the increased social behaviour of pairs of male rats induced by short term isolation. *Neuropharmacology, 21*(12), 1343-1348. http://dx.doi.org/10.1016/0028-3908(82)90144-7

Niesink, R. J., & Van Ree, J. M. (1982b). Short-term isolation increases social interactions of male rats: a parametric analysis. *Physiol Behav., 29*(5), 819-825. http://dx.doi.org/10.1016/0031-9384(82)90331-6

Nixon, J. P., Zhang, M., Wang, C., Kuskowski, M. A., Novak, C. M., Levine, J. A. et al. (2010). Evaluation of a quantitative magnetic resonance imaging system for whole body composition analysis in rodents. *Obesity.(Silver.Spring), 18*(8), 1652-1659. doi: 10.1038/oby.2009.471

Novick, A. M., Miller, L. C., Forster, G. L., & Watt, M. J. (2013). Adolescent social defeat decreases spatial working memory performance in adulthood. *Behav.Brain Funct., 9*, 39. doi: 10.1186/1744-9081-9-39

O'Connor, D. B., Jones, F., Conner, M., McMillan, B., & Ferguson, E. (2008). Effects of daily hassles and eating style on eating behavior. *Health Psychol., 27*(1 Suppl), S20-S31. http://dx.doi.org/10.1037/0278-6133.27.1.s20

Ong, Z. Y., Wanasuria, A. F., Lin, M. Z., Hiscock, J., & Muhlhausler, B. S. (2013). Chronic intake of a cafeteria diet and subsequent abstinence. Sex-specific effects on gene expression in the mesolimbic reward system. *Appetite, 65*, 189-199. doi: 10.1016/j.appet.2013.01.014

Ortolani, D., Garcia, M. C., Melo-Thomas, L., & Spadari-Bratfisch, R. C. (2014). Stress-induced endocrine response and anxiety: the effects of comfort food in rats. *Stress., 17*(3), 211-218. doi:10.3109/10253890.2014.898059

Ossowska, G., Danilczuk, Z., Klenk-Majewska, B., Czajkowski, L., & Zebrowska-Lupina, I. (2004). Antidepressants in chronic unpredictable mild stress (CUMS)-induced deficit of fighting behavior. *Pol.J.Pharmacol., 56*(3), 305-311. http://if-pan.krakow.pl/pjp/

Ostrander, M. M., Ulrich-Lai, Y. M., Choi, D. C., Richtand, N. M., & Herman, J. P. (2006). Hypoactivity of the hypothalamo-pituitary-adrenocortical axis during recovery from chronic variable stress. *Endocrinology, 147*(4), 2008-2017. doi: 10.1210/en.2005-1041

Overmier, J. B., & Murison, R. (1991). Juvenile and adult footshock stress modulate later adult gastric pathophysiological reactions to restraint stresses in rats. *Behav.Neurosci., 105*(2), 246-252. http://dx.doi.org/10.1037/0735-7044.105.2.246

Oztan, O., Aydin, C., & Isgor, C. (2011). Chronic variable physical stress during the peripubertal-juvenile period causes differential depressive and anxiogenic effects in the novelty-seeking phenotype: functional implications for hippocampal and amygdalar brain-derived neurotrophic factor and the mossy fibre plasticity. *Neuroscience, 192*, 334-344. doi: 10.1016/j.neuroscience.2011.06.077

Packard, A. E., Ghosal, S., Herman, J. P., Woods, S. C., & Ulrich-Lai, Y. M. (2014). Chronic variable stress improves glucose tolerance in rats with sucrose-induced prediabetes. *Psychoneuroendocrinology, 47*, 178-188. doi: 10.1016/j.psyneuen.2014.05.016

Padovan, C. M., & Guimaraes, F. S. (2000). Restraint-induced hypoactivity in an elevated plus-maze. *Braz.J.Med.Biol.Res., 33*(1), 79-83. http://dx.doi.org/10.1590/s0100-879x2000000100011

Pan, Y., Wang, F. M., Qiang, L. Q., Zhang, D. M., & Kong, L. D. (2010). Icariin attenuates chronic mild stress-induced dysregulation of the LHPA stress circuit in rats. *Psychoneuroendocrinology, 35*(2), 272-283. doi: 10.1016/j.psyneuen.2009.06.020

Panksepp, J. (1981). The ontogeny of play in rats. *Dev.Psychobiol., 14*(4), 327-332. http://dx.doi.org/10.1002/dev.420140405

Panksepp, J., Burgdorf, J., Beinfeld, M. C., Kroes, R. A., & Moskal, J. R. (2007). Brain regional neuropeptide changes resulting from social defeat. *Behav.Neurosci., 121*(6), 1364-1371. doi: 10.1037/0735-7044.121.6.1364

Papciak, J., Popik, P., Fuchs, E., & Rygula, R. (2013). Chronic psychosocial stress makes rats more 'pessimistic' in the ambiguous-cue interpretation paradigm. *Behav.Brain Res., 256*, 305-310. doi: 10.1016/j.bbr.2013.08.036

Papp, M., Lappas, S., Muscat, R., & Willner, P. (1992). Attenuation of place preference conditioning but not place aversion conditioning by chronic mild stress. *J.Psychopharmacol., 6*(3), 352-356. http://dx.doi.org/10.1177/026988119200600302

Papp, M., Moryl, E., & Willner, P. (1996). Pharmacological validation of the chronic mild stress model of depression. *European Journal of Pharmacology, 296*(2), 129-136. doi: 10.1016/0014-2999(95)00697-4

Papp, M., Willner, P., & Muscat, R. (1991). An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. *Psychopharmacology (Berl), 104*(2), 255-259. http://dx.doi.org/10.1007/bf02244188

Pardon, M., Gerardin, P., Joubert, C., Perez-Diaz, F., & Cohen-Salmon, C. (2000). Influence of prepartum chronic ultramild stress on maternal pup care behavior in mice. *Biological Psychiatry, 47*(10), 858-863. http://dx.doi.org/10.1016/s0006-3223(99)00253-x

Park, E., Kim, J. Y., Lee, J.-H., & Jhang, J. W. (2014). Increased depression-like behaviors with dysfunctions in the stress axis and the reward center by free access to highly palatable food. *Neuroscience, 262*, 31-29. http://dx.doi.org/10.1016/j.neuroscience.2013.12.054

Parker, V., & Morinan, A. (1986). The socially-isolated rat as a model for anxiety. *Neuropharmacology, 25*, 663-664. http://dx.doi.org/10.1016/0028-3908(86)90224-8

Paternain, L., Garcia-Diaz, D. F., Milagro, F. I., Gonzalez-Muniesa, P., Martinez, J. A., & Campion, J. (2011). Regulation by chronic-mild stress of glucocorticoids, monocyte chemoattractant protein-1 and adiposity in rats fed on a high-fat diet. *Physiol Behav., 103*(2), 173-180. doi: 10.1016/j.physbeh.2011.01.017

Patki, G., Solanki, N., Atrooz, F., Allam, F., & Salim, S. (2013). Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress. *Brain Research, 1539*, 73-86. doi: 10.1016/j.brainres.2013.09.033

Patterson, Z. R., & Abizaid, A. (2013). Stress induced obesity: lessons from rodent models of stress. *Front Neurosci., 7*, 130. doi:10.3389/fnins.2013.00130

Patterson, Z. R., Ducharme, R., Anisman, H., & Abizaid, A. (2010). Altered metabolic and neurochemical responses to chronic unpredictable stressors in ghrelin receptor-deficient mice. *European Journal of Neuroscience, 32*(4), 632-639. doi: 10.1111/j.1460-9568.2010.07310.x

Patterson, Z. R., Khazall, R., Mackay, H., Anisman, H., & Abizaid, A. (2013). Central ghrelin signaling mediates the metabolic response of C57BL/6 male mice to chronic social defeat stress. *Endocrinology, 154*(3), 1080-1091. doi: 10.1210/en.2012-1834

Patterson, Z. R., Parno, T., Isaacs, A. M., & Abizaid, A. (2013). Interruption of ghrelin signaling in the PVN increases high-fat diet intake and body weight in stressed and non-stressed C57BL6J male mice. *Front Neurosci., 7*, 167. doi:10.3389/fnins.2013.00167

Paul, E. D., Hale, M. W., Lukkes, J. L., Valentine, M. J., Sarchet, D. M., & Lowry, C. A. (2011). Repeated social defeat increases reactive emotional coping behavior and alters functional responses in serotonergic neurons in the rat dorsal raphe nucleus. *Physiol Behav., 104*(2), 272-282. doi: 10.1016/j.physbeh.2011.01.006

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates* (4th ed.). New York: Academic Press.

Paylor, R., Spencer, C. M., Yuva-Paylor, L. A., & Pieke-Dahl, S. (2006). The use of behavioral test batteries, II: effect of test interval. *Physiol Behav., 87*(1), 95-102. doi: 10.1016/j.physbeh.2005.09.002

Pecoraro, N., Reyes, F., Gomez, F., Bhargava, A., & Dallman, M. F. (2004). Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress. *Endocrinology, 145*(8), 3754-3762. http://dx.doi.org/10.1210/en.2004-0305

Pellis, S. M., Field, E. F., & Whishaw, I. Q. (1999). The development of a sex-differentiated defensive motor pattern in rats: A possible role for juvenile experience. *Dev.Psychobiol., 35*(2), 156-164. http://dx.doi.org/10.1002/(sici)1098-2302(199909)35:2<156::aid-dev8>3.0.co;2-c

Pellis, S., & Pellis, V. (2007). Rough-and-tumble play and the development of the social brain. *Curr Dir Psychol Sci, 16*(2), 95-98. http://dx.doi.org/10.1111/j.1467-8721.2007.00483.x

Perlman, W. R., Webster, M. J., Herman, M. M., Kleinman, J. E., & Weickert, C. S. (2007). Age-related differences in glucocorticoid receptor mRNA levels in the human brain. *Neurobiol.Aging, 28*(3), 447-458. doi: 10.1016/j.neurobiolaging.2006.01.010

Pervanidou, P., & Chrousos, G. P. (2011). Stress and obesity/metabolic syndrome in childhood and adolescence. *Int.J.Pediatr.Obes., 6 Suppl 1*, 21-28. doi: 10.3109/17477166.2011.615996

Pinhas-Hamiel, O., & Zeitler, P. (1996). Insulin resistance, obesity, and related disorders among black adolescents. *Journal of Pediatrics, 129*(3), 319-320. http://dx.doi.org/10.1016/s0022-3476(96)70060-4

Pliner, P., & Mann, N. (2004). Influence of social norms and palatability on amount consumed and food choice. *Appetite, 42*(2), 227-237. http://dx.doi.org/10.1016/j.appet.2003.12.001

Pohl, J., Olmstead, M. C., Wynne-Edwards, K. E., Harkness, K., & Menard, J. L. (2007). Repeated exposure to stress across the childhood-adolescent period alters rats' anxiety- and depression-like behaviors in adulthood: The importance of stressor type and gender. *Behav.Neurosci., 121*(3), 462-474. doi: 10.1037/0735-7044.121.3.462

Poirier, G. L., Imamura, N., Zanoletti, O., & Sandi, C. (2014). Social deficits induced by peripubertal stress in rats are reversed by resveratrol. *Journal of Psychiatric Research, 57*, 157-164. doi: 10.1016/j.jpsychires.2014.05.017

Popkin, B. M., Duffey, K., & Gordon-Larsen, P. (2005). Environmental influences on food choice, physical activity and energy balance. *Physiol Behav., 86*(5), 603-613. http://dx.doi.org/10.1016/j.physbeh.2005.08.051 Porsolt, R. D., Anton, G., Blavet, N., & Jalfre, M. (1978). Behavioural despair in rats: a new model sensitive to antidepressant treatments. *European Journal of Pharmacology, 47*(4), 379-391. http://dx.doi.org/10.1016/0014-2999(78)90118-8

Porsolt, R. D., Bertin, A., & Jalfre, M. (1977). Behavioral despair in mice: a primary screening test for antidepressants. *Archives Internationales de Pharmacodynamie et de Therapie, 229*(2), 327-336. Retrieved from PM:596982

Porsolt, R. D., Le Pichon, M., & Jalfre, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature, 266*(5604), 730-732. http://dx.doi.org/10.1038/266730a0

Pou, K. M., Massaro, J. M., Hoffmann, U., Lieb, K., Vasan, R. S., O'Donnell, C. J. et al. (2009). Patterns of abdominal fat distribution: the Framingham Heart Study. *Diabetes Care, 32*(3), 481-485. doi: 10.2337/dc08-1359

Pucilowski, O., Overstreet, D. H., Rezvani, A. H., & Janowsky, D. S. (1993). Chronic mild stress-induced anhedonia: Greater effect in a genetic rat model of depression. *Physiol.Behav., 54*, 1215-1220. http://dx.doi.org/10.1016/0031-9384(93)90351-f

Pulliam, J. V., Dawaghreh, A. M., Alema-Mensah, E., & Plotsky, P. M. (2010). Social defeat stress produces prolonged alterations in acoustic startle and body weight gain in male

Long Evans rats. *Journal of Psychiatric Research, 44*(2), 106-111. doi: 10.1016/j.jpsychires.2009.05.005

Randle, P. J. (1998). Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab Rev., 14*(4), 263-283. http://dx.doi.org/10.1002/(sici)1099-0895(199812)14:4<263::aid-dmr233>3.0.co;2-c

Ratka, A., Sutanto, W., Bloemers, M., & de Kloet, E. R. (1989). On the role of brain mineralocorticoid (type I) and glucocorticoid (type II) receptors in neuroendocrine regulation. *Neuroendocrinology, 50*(2), 117-123. http://dx.doi.org/10.1159/000125210

Razzoli, M., Carboni, L., Guidi, A., Gerrard, P., & Arban, R. (2007). Social defeat-induced contextual conditioning differentially imprints behavioral and adrenal reactivity: a time-course study in the rat. *Physiol Behav., 92*(4), 734-740. doi: 10.1016/j.physbeh.2007.05.063

Reul, J. M., & de Kloet, E. R. (1985). Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology, 117*(6), 2505-2511. doi:10.1210/endo-117-6-2505

Reul, J. M., & de Kloet, E. R. (1986). Anatomical resolution of two types of corticosterone receptor sites in rat brain with in vitro autoradiography and computerized image analysis. *Journal of Steroid Biochemistry, 24*(1), 269-272. http://dx.doi.org/10.1016/0022-4731(86)90063-4

Robbins, T. W., Jones, G. H., & Wilkinson, L. S. (1996). Behavioural and neurochemical effects of early social deprivation in the rat. *J.Psychopharmacol., 10*(1), 39-47. doi: 10.1177/026988119601000107

Rockman, G. E., Hall, A., Markert, L., & Glavin, G. B. (1987). Early weaning effects on voluntary ethanol consumption and stress responsivity in rats. *Physiol Behav., 40*(5), 673-676. http://dx.doi.org/10.1016/0031-9384(87)90116-8

Rodgers, R. J., Haller, J., Holmes, A., Halasz, J., Walton, T. J., & Brain, P. F. (1999). Corticosterone response to the plus-maze: high correlation with risk assessment in rats and mice. *Physiol Behav., 68*(1-2), 47-53. http://dx.doi.org/10.1016/s0031-9384(99)00140-7

Romeo, R. D. (2010). Adolescence: a central event in shaping stress reactivity. *Dev.Psychobiol., 52*, 244-253. http://dx.doi.org/10.1002/dev.20437

Romeo, R. D., Bellani, R., Karatsoreos, I. N., Chhua, N., Vernov, M., Conrad, C. D. et al. (2006). Stress history and pubertal development interact to shape hypothalamic-pituitary-adrenal axis plasticity. *Endocrinology, 147*(4), 1664-1674. doi: 10.1210/en.2005-1432

Romeo, R. D., Karatsoreos, I. N., & McEwen, B. S. (2006). Pubertal maturation and time of day differentially affect behavioral and neuroendocrine responses following an acute stressor. *Horm.Behav., 50*(3), 463-468. doi: 10.1016/j.yhbeh.2006.06.002

Romeo, R. D., Lee, S. J., Chhua, N., McPherson, C. R., & McEwen, B. S. (2004). Testosterone cannot activate an adult-like stress response in prepubertal male rats. *Neuroendocrinology, 79*(3), 125-132. doi:10.1159/000077270

Romeo, R. D., Lee, S. J., & McEwen, B. S. (2004). Differential stress reactivity in intact and ovariectomized prepubertal and adult female rats. *Neuroendocrinology, 80*(6), 387-393. doi:10.1159/000084203

Rossler, A. S., Joubert, C., & Chapouthier, G. (2000). Chronic mild stress alleviates anxious behaviour in female mice in two situations. *Behav.Processes, 49*(3), 163-165. http://dx.doi.org/10.1016/s0376-6357(00)00080-2

Rubin, R. T., Mandell, A. J., & Crandall, P. H. (1966). Corticosteroid responses to limbic stimulation in man: localization of stimulus sites. *Science, 153*(3737), 767-768. http://dx.doi.org/10.1126/science.153.3737.767

Ruis, M. A., te Brake, J. H., Buwalda, B., De Boer, S. F., Meerlo, P., Korte, S. M. et al. (1999). Housing familiar male wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat. *Psychoneuroendocrinology, 24*(3), 285-300. http://dx.doi.org/10.1016/s0306-4530(98)00050-x

Rygula, R., Abumaria, N., Flugge, G., Fuchs, E., Ruther, E., Havemann-Reinecke, U. et al. (2005). Anhedonia and motivational deficits in rats: impact of chronic social stress. *Behavioural Brain Research, 162*(1), 127-134. http://dx.doi.org/10.1016/j.bbr.2005.03.009

Rygula, R., Abumaria, N., Havemann-Reinecke, U., Ruther, E., Hiemke, C., Zernig, G. et al. (2008). Pharmacological validation of a chronic social stress model of depression in rats: effects of reboxetine, haloperidol and diazepam. *Behavioural Pharmacology, 19*(3), 183-196. doi:10.1097/FBP.0b013e3282fe8871

Sachser, N., Durschlag, M., & Hirzel, D. (1998). Social relationships and the management of stress. *Psychoneuroendocrinology, 23*(8), 891-904. http://dx.doi.org/10.1016/S0306-4530(98)00059-6

Said, N., Lakehayli, S., El, K. M., El, O. M., Nadifi, S., Hakkou, F. et al. (2015). Prenatal stress induces vulnerability to nicotine addiction and alters D2 receptors' expression in the nucleus accumbens in adult rats. *Neuroscience, 304*, 279-285. doi: 10.1016/j.neuroscience.2015.07.029

Sampson, D., Muscat, R., Phillips, G., & Willner, P. (1992). Decreased reactivity to sweetness following chronic exposure to mild unpredictable stress or acute administration of pimozide. *Neuroscience & Biobehavioral Reviews, 16*, 519-524. http://dx.doi.org/10.1016/s0149-7634(05)80193-9

Sampson, D., Willner, P., & Muscat, R. (1991). Reversal of antidepressant action by dopamine antagonists in an animal model of depression. *Psychopharmacology (Berl), 104*(4), 491-495. http://dx.doi.org/10.1007/bf02245655

Sapolsky, R. M. (1982). The endocrine stress-response and social status in the wild baboon. *Horm.Behav., 16*(3), 279-292. http://dx.doi.org/10.1016/0018-506x(82)90027-7

Sapolsky, R. M. (1983). Individual differences in cortisol secretory patterns in the wild baboon: role of negative feedback sensitivity. *Endocrinology, 113*(6), 2263-2267. doi:10.1210/endo-113-6-2263

Sapolsky, R. M., Meaney, M. J., & McEwen, B. S. (1985). The development of the glucocorticoid receptor system in the rat limbic brain. III. Negative-feedback regulation. *Brain Research, 350*(1-2), 169-173. http://dx.doi.org/10.1016/0165-3806(85)90261-5

Saul, M. L., Tylee, D., Becoats, K. T., Guerrero, B. G., Sweeney, P., Helmreich, D. L. et al. (2012). Long-term behavioral consequences of stress exposure in adolescent versus young adult rats. *Behav.Brain Res., 229*(1), 226-234. doi: 10.1016/j.bbr.2012.01.022

Sawchenko, P. E. (1987). Evidence for a local site of action for glucocorticoids in inhibiting CRF and vasopressin expression in the paraventricular nucleus. *Brain Research, 403*(2), 213-223. http://dx.doi.org/10.1016/0006-8993(87)90058-8

Scaccianoce, S., Del, B. P., Paolone, G., Caprioli, D., Modafferi, A. M., Nencini, P. et al. (2006). Social isolation selectively reduces hippocampal brain-derived neurotrophic factor without altering plasma corticosterone. *Behav.Brain Res., 168*(2), 323-325. doi: 10.1016/j.bbr.2005.04.024

Schifter, S. (1991). Circulating concentrations of calcitonin gene-related peptide (CGRP) in normal man determined with a new, highly sensitive radioimmunoassay. *Peptides, 12*, 365-369. http://dx.doi.org/10.1016/0196-9781(91)90027-m

Schiller, G. D., Pucilowski, O., Wienicke, C., & Overstreet, D. H. (1992). Immobility-reducing effects of antidepressants in a genetic animal model of depression. *Brain Res Bull, 28*(5), 821-823. http://dx.doi.org/10.1016/0361-9230(92)90267-2

Schmidt, M. V., Czisch, M., Sterlemann, V., Reinel, C., Samann, P., & Muller, M. B. (2009). Chronic social stress during adolescence in mice alters fat distribution in late life: prevention by antidepressant treatment. *Stress., 12*(1), 89-94. http://dx.doi.org/10.1080/10253890802049343

Schmidt, M. V., Scharf, S. H., Sterlemann, V., Ganea, K., Liebl, C., Holsboer, F. et al. (2010). High susceptibility to chronic social stress is associated with a depression-like phenotype. *Psychoneuroendocrinology, 35*(5), 635-643. doi: 10.1016/j.psyneuen.2009.10.002

Schmidt, M. V., Sterlemann, V., Ganea, K., Liebl, C., Alam, S., Harbich, D. et al. (2007). Persistent neuroendocrine and behavioral effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence. *Psychoneuroendocrinology, 32*(5), 417-429. doi: 10.1016/j.psyneuen.2007.02.011

Schrijver, N. C., Bahr, N. I., Weiss, I. C., & Wurbel, H. (2002). Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats. *Pharmacology, Biochemistry and Behavior, 73*(1), 209-224. http://dx.doi.org/10.1016/s0091-3057(02)00790-6

Schweizer, M. C., Henniger, M. S., & Sillaber, I. (2009). Chronic mild stress (CMS) in mice: of anhedonia, 'anomalous anxiolysis' and activity. *PLoS.One., 4*(1), e4326. doi:10.1371/journal.pone.0004326

Scott, K. A., Melhorn, S. J., & Sakai, R. R. (2012). Effects of Chronic Social Stress on Obesity. *Curr.Obes.Rep., 1*(1), 16-25. doi:10.1007/s13679-011-0006-3

Secoli, S. R., & Teixeira, N. A. (1998). Chronic prenatal stress affects development and behavioral depression in rats. *Stress., 2*(4), 273-280. http://dx.doi.org/10.3109/10253899809167291

Seidell, J. C. (1998). Dietary fat and obesity: an epidemiologic perspective. *American Journal of Clinical Nutrition, 67*(3 Suppl), 546S-550S. http://ajcn.nutrition.org/

Serra, M., Pisu, M. G., Floris, I., & Biggio, G. (2005). Social isolation-induced changes in the hypothalamic-pituitary-adrenal axis in the rat. *Stress., 8*(4), 259-264. doi: 10.1080/10253890500495244

Shaibi, G. Q., & Goran, M. I. (2008). Examining metabolic syndrome definitions in overweight Hispanic youth: a focus on insulin resistance. *Journal of Pediatrics, 152*(2), 171-176. http://dx.doi.org/10.1016/j.jpeds.2007.08.010

Shimai, S., Kawabata, T., Nishioka, N., & Haruki, T. (2000). [Snacking behavior among elementary and junior high school students and its relationship to stress-coping]. *Nihon Koshu Eisei Zasshi, 47*(1), 8-19. https://www.jstage.jst.go.jp/browse/jph/

Shively, C., & Kaplan, J. (1984). Effects of social factors on adrenal weight and related physiology of Macaca fascicularis. *Physiol Behav., 33*(5), 777-782. http://dx.doi.org/10.1016/0031-9384(84)90047-7

Shively, C. A., Laber-Laird, K., & Anton, R. F. (1997). Behavior and physiology of social stress and depression in female cynomolgus monkeys. *Biological Psychiatry, 41*(8), 871-882. doi:.1016/S0006-3223(96)00185-0

Sigman, G. S. (2003). Eating disorders in children and adolescents. *Pediatric Clinics of North America, 50*(5), 1139-77, doi:10.1016/S0031-3955(03)00067-1

Sikiric, P., Separovic, J., Buljat, G., Anic, T., Stancic-Rokotov, D., Mikus, D. et al. (2000). The antidepressant effect of an antiulcer pentadecapeptide BPC 157 in Porsolt's test and chronic unpredictable stress in rats. A comparison with antidepressants. *J.Physiol Paris, 94*(2), 99-104. http://dx.doi.org/10.1016/s0928-4257(00)00148-0

Silberman, D. M., Wald, M., & Genaro, A. M. (2002). Effects of chronic mild stress on lymphocyte proliferative response. Participation of serum thyroid hormones and corticosterone. *Int.Immunopharmacol., 2*(4), 487-497. http://dx.doi.org/10.1016/s1567-5769(01)00190-4

Simon, P., Dupuis, R., & Costentin, J. (1994). Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. *Behav.Brain Res., 61*(1), 59-64. http://dx.doi.org/10.1016/0166-4328(94)90008-6

Singh, A. S., Mulder, C., Twisk, J. W., van Mechelen, W., & Chinapaw, M. J. (2008). Tracking of childhood overweight into adulthood: a systematic review of the literature. *Obes.Rev., 9*(5), 474-488. http://dx.doi.org/10.1111/j.1467-789x.2008.00475.x

Skelton, J. A., Cook, S. R., Auinger, P., Klein, J. D., & Barlow, S. E. (2009). Prevalence and trends of severe obesity among US children and adolescents. *Acad.Pediatr., 9*(5), 322-329. http://dx.doi.org/10.1016/j.acap.2009.04.005

Slattery, D. A., & Cryan, J. F. (2012). Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nature Protocols, 7*(6), 1009-1014. http://dx.doi.org/10.1038/nprot.2012.044

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience 8*, 383-395. http://www.dialogues-cns.com/

Solomon, M. B., Foster, M. T., Bartness, T. J., & Huhman, K. L. (2007). Social defeat and footshock increase body mass and adiposity in male Syrian hamsters. *Am.J.Physiol Regul.Integr.Comp Physiol, 292*(1), R283-R290. doi: 10.1152/ajpregu.00330.2006

Solomon, M. B., Jankord, R., Flak, J. N., & Herman, J. P. (2011). Chronic stress, energy balance and adiposity in female rats. *Physiol Behav., 102*(1), 84-90. doi: 10.1016/j.physbeh.2010.09.024

Song, L., Che, W., Min-Wei, W., Murakami, Y., & Matsumoto, K. (2006). Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. *Pharmacology, Biochemistry and Behavior, 83*(2), 186-193. doi: 10.1016/j.pbb.2006.01.004

Soulis, G., Papalexi, E., Kittas, C., & Kitraki, E. (2007). Early impact of a fat-enriched diet on behavioral responses of male anf female rats. *Behavioral Neuroscience, 121*, 483-490. http://dx.doi.org/10.1037/0735-7044.121.3.483

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience & Biobehavioral Reviews, 24*(4), 417-463. http://dx.doi.org/10.1016/s0149-7634(00)00014-2

Spriijt-Metz, D. (2011). Etiology, treatment, and prevention of obesity in childhood and adolescence: A decade in review. *Journal of Reserach on Adolescence, 21*(1), 129-152. http://dx.doi.org/10.1111/j.1532-7795.2010.00719.x

Spruijt, B. M., Van Hooff, J. A. R. A. M., & Gispen, W. H. (1992). Ethology and neurobiology of grooming behavior. *Physiological Reviews, 72*, 825-852. http://physrev.physiology.org/

Stansfield, K. H., & Kirstein, C. L. (2006). Effects of novelty on behavior in the adolescent and adult rat. *Dev.Psychobiol., 48*(1), 10-15. doi:10.1002/dev.20127

Statistics Canada. (2011). Adult obesity prevalence in Canada and the United States. *Statistics Canada*. Retrieved from http://www.statcan.gc.ca/pub/82-625-x/2011001/article/11411-eng.htm

Statistics Canada. (2012a). Body mass index, overweight or obese, self-reported, adult, by age group and sex. *Statistics Canada*. Retrieved from http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/health81b-eng.htm

Statistics Canada. (2012b). Body mass index, overweight or obese, self-reported, youth, by sex, provinces and territories. *Statistics Canada*. Retrieved from http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/health84b-eng.htm

Statistics Canada. (2012c). Overweight and obesity in children and adolescents: Results from the 2009 to 2011 Canadian Health Measures Survey. *Statistics Canada*. Retrieved from http://www.statcan.gc.ca/pub/82-003-x/2012003/article/11706-eng.htm

Stein, C. J., & Colditz, G. A. (2004). The epidemic of obesity. *J.Clin.Endocrinol.Metab, 89*(6), 2522-2525. http://press.endocrine.org/journal/jcem

Steptoe, A., Feldman, P. J., Kunz, S., Owen, N., Willemsen, G., & Marmot, M. (2002). Stress responsivity and socioeconomic status: a mechanism for increased cardiovascular disease risk? *Eur.Heart J., 23*(22), 1757-1763. http://dx.doi.org/10.1053/euhj.2001.3233

Sterlemann, V., Ganea, K., Liebl, C., Harbich, D., Alam, S., Holsboer, F. et al. (2008). Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: implications for stress-related disorders. *Horm.Behav., 53*(2), 386-394. doi: 10.1016/j.yhbeh.2007.11.001

Strack, A. M., Akana, S. F., Horsley, C. J., & Dallman, M. F. (1997). A hypercaloric load induces thermogenesis but inhibits stress responses in the SNS and HPA system. *Am.J.Physiol, 272*(3 Pt 2), R840-R848. http://www.physiology.org/

Strekalova, T., Spanagel, R., Bartsch, D., Henn, F. A., & Gass, P. (2004). Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. *Neuropsychopharmacology, 29*(11), 2007-2017. doi:10.1038/sj.npp.1300532

Stunkard, A. J., Faith, M. S., & Allison, K. C. (2003). Depression and obesity. *Biological Psychiatry, 54*(3), 330-337. http://www.journals.elsevier.com/biological-psychiatry/

Tamashiro, K. L., Nguyen, M. M., Fujikawa, T., Xu, T., Yun, M. L., Woods, S. C. et al. (2004). Metabolic and endocrine consequences of social stress in a visible burrow system. *Physiol Behav., 80*(5), 683-693. doi:10.1016/j.physbeh.2003.12.002

Tamashiro, K. L., Nguyen, M. M., & Sakai, R. R. (2005). Social stress: from rodents to primates. *Front Neuroendocrinol., 26*(1), 27-40. doi: 10.1016/j.yfrne.2005.03.001

Tamashiro, K. L., Sakai, R. R., Shively, C. A., Karatsoreos, I. N., & Reagan, L. P. (2011). Chronic stress, metabolism, and metabolic syndrome. *Stress., 14*(5), 468-474. http://dx.doi.org/10.3109/10253890.2011.606341

Tannenbaum, B. M., Brindley, D. N., Tannenbaum, G. S., Dallman, M. F., McArthur, M. D., & Meaney, M. J. (1997). High-fat feeding alters both basal and stress-induced hypothalamic-pituitary-adrenal activity in the rat. *Am.J.Physiol, 273*(6 Pt 1), E1168-E1177. http://www.physiology.org/

Taylor, J. R. (1997). *An Introduction to Error Analysis* (2 ed.). Sausolito, California: University Science Books.

Taylor, S. E., Klein, L. C., Lewis, B. P., Gruenewald, T. L., Gurung, R. A., & Updegraff, J. A. (2000). Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychol.Rev., 107*(3), 411-429. http://dx.doi.org/10.1037/0033-295x.107.3.411

Thorsell, A., Slawecki, C. J., El, K. A., Mathe, A. A., & Ehlers, C. L. (2006). The effects of social isolation on neuropeptide Y levels, exploratory and anxiety-related behaviors in rats. *Pharmacology, Biochemistry and Behavior, 83*(1), 28-34. doi: 10.1016/j.pbb.2005.12.005

Tidey, J. W., & Miczek, K. A. (1996). Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. *Brain Research, 721*(1-2), 140-149. http://dx.doi.org/10.1016/0006-8993(96)00159-x

Tirelli, E., Laviola, G., & Adriani, W. (2003). Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. *Neuroscience & Biobehavioral Reviews, 27*(1-2), 163-178. http://dx.doi.org/10.1016/s0149-7634(03)00018-6

Toledo-Rodriguez, M., & Sandi, C. (2007). Stress before puberty exerts a sex- and age-related impact on auditory and contextual fear conditioning in the rat. *Neural Plast., 2007*, 71203. http://dx.doi.org/10.1155/2007/71203

Torres, S. J., & Nowson, C. A. (2007). Relationship between stress, eating behavior, and obesity. *Nutrition, 23*(11-12), 887-894. http://dx.doi.org/10.1016/j.nut.2007.08.008

Toth, E., Avital, A., Leshem, M., Richter-Levin, G., & Braun, K. (2008). Neonatal and juvenile stress induces changes in adult social behavior without affecting cognitive function. *Behav.Brain Res., 190*(1), 135-139. http://dx.doi.org/10.1016/j.bbr.2008.02.012

Toth, E., Gersner, R., Wilf-Yarkoni, A., Raizel, H., Dar, D. E., Richter-Levin, G. et al. (2008). Age-dependent effects of chronic stress on brain plasticity and depressive behavior. *J.Neurochem., 107*(2), 522-532. doi: 10.1111/j.1471-4159.2008.05642.x

Toth, M., Mikics, E., Tulogdi, A., Aliczki, M., & Haller, J. (2011). Post-weaning social isolation induces abnormal forms of aggression in conjunction with increased glucocorticoid and autonomic stress responses. *Horm.Behav., 60*(1), 28-36. doi: 10.1016/j.yhbeh.2011.02.003

Treit, D., & Fundytus, M. (1988). Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacology, Biochemistry and Behavior, 31*(4), 959-962. http://dx.doi.org/10.1016/0091-3057(88)90413-3

Tsankova, N. M., Berton, O., Renthal, W., Kumar, A., Neve, R. L., & Nestler, E. J. (2006). Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nature Neuroscience, 9*(4), 519-525. doi: 10.1038/nn1659

Tsoory, M., Cohen, H., & Richter-Levin, G. (2007). Juvenile stress induces a predisposition to either anxiety or depressive-like symptoms following stress in adulthood. *European Neuropsychopharmacology, 17*(4), 245-256. http://dx.doi.org/10.1016/j.euroneuro.2006.06.007

Tsoory, M., & Richter-Levin, G. (2006). Learning under stress in the adult rat is differentially affected by 'juvenile' or 'adolescent' stress. *Int.J.Neuropsychopharmacol., 9*(6), 713-728. http://dx.doi.org/10.1017/s1461145705006255

Ulrich-Lai, Y. M., Christiansen, A. M., Ostrander, M. M., Jones, A. A., Jones, K. R., Choi, D. C. et al. (2010). Pleasurable behaviors reduce stress via brain reward pathways. *Proc.Natl.Acad.Sci.U.S.A, 107*(47), 20529-20534. http://dx.doi.org/10.1073/pnas.1007740107

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nat.Rev.Neurosci., 10*(6), 397-409. http://dx.doi.org/10.1038/nrn2647

Ulrich-Lai, Y. M., Ostrander, M. M., & Herman, J. P. (2011). HPA axis dampening by limited sucrose intake: reward frequency vs. caloric consumption. *Physiol Behav., 103*(1), 104-110. http://dx.doi.org/10.1016/j.physbeh.2010.12.011

Ulrich-Lai, Y. M., Ostrander, M. M., Thomas, I. M., Packard, B. A., Furay, A. R., Dolgas, C. M. et al. (2007). Daily limited access to sweetened drink attenuates hypothalamic-pituitary-adrenocortical axis stress responses. *Endocrinology, 148*(4), 1823-1834. http://dx.doi.org/10.1210/en.2006-1241

Uno, H., Tarara, R., Else, J. G., Suleman, M. A., & Sapolsky, R. M. (1989). Hippocampal damage associated with prolonged and fatal stress in primates. *Journal of Neuroscience, 9*(5), 1705-1711. http://www.jneurosci.org/

Unterwald, E. M., Ho, A., Rubenfeld, J. M., & Kreek, M. J. (1994). Time course of the development of behavioral sensitization and dopamine receptor up-regulation during binge cocaine administration. *Journal of Pharmacology & Experimental Therapeutics, 270*(3), 1387-1396. http://jpet.aspetjournals.org/

Uys, J. D., Marais, L., Faure, J., Prevoo, D., Swart, P., Mohammed, A. H. et al. (2006a). Developmental trauma is associated with behavioral hyperarousal, altered HPA axis activity, and decreased hippocampal neurotrophin expression in the adult rat. *Ann.N.Y.Acad.Sci, 1071*, 542-546. doi: 10.1196/annals.1364.060

Uys, J. D., Muller, C. J., Marais, L., Harvey, B. H., Stein, D. J., & Daniels, W. M. (2006b). Early life trauma decreases glucocorticoid receptors in rat dentate gyrus upon adult re-stress: reversal by escitalopram. *Neuroscience, 137*(2), 619-625. doi: 10.1016/j.neuroscience.2005.08.089

Valverde, O., Smadja, C., Roques, B. P., & Maldonado, R. (1997). The attenuation of morphine-conditioned place preference following chronic mild stress is reversed by a CCKB receptor antagonist. *Psychopharmacology (Berl), 131*(1), 79-85. http://dx.doi.org/10.1007/s002130050268

Valzelli, L. (1973). The "isolation syndrome" in mice. *Psychopharmacologia., 31*(4), 305-320. http://dx.doi.org/10.1007/bf00421275

van den Berg, C. L., Hol, T., Van Ree, J. M., Spruijt, B. M., Everts, H., & Koolhaas, J. M. (1999). Play is indispensable for an adequate development of coping with social

challenges in the rat. *Dev.Psychobiol., 34*(2), 129-138. http://dx.doi.org/10.1002/(sici)1098-2302(199903)34:2<129::aid-dev6>3.0.co;2-l

van, W. R., Stefanis, N. C., & Myin-Germeys, I. (2008). Psychosocial stress and psychosis. A review of the neurobiological mechanisms and the evidence for gene-stress interaction. *Schizophrenia Bulletin, 34*(6), 1095-1105. doi: 10.1093/schbul/sbn101

Vazquez, D. M. (1998). Stress and the developing limbic-hypothalamic-pituitary-adrenal axis. *Psychoneuroendocrinology, 23*(7), 663-700. http://dx.doi.org/10.1016/s0306-4530(98)00029-8

Vazquez, D. M., & Akil, H. (1993). Pituitary-adrenal response to ether vapor in the weanling animal: characterization of the inhibitory effect of glucocorticoids on adrenocorticotropin secretion. *Pediatr.Res., 34*(5), 646-653. doi:10.1203/00006450-199311000-00017

Veenema, A. H. (2009). Early life stress, the development of aggression and neuroendocrine and neurobiological correlates: what can we learn from animal models? *Front Neuroendocrinol., 30*(4), 497-518. doi: 10.1016/j.yfrne.2009.03.003

Ver Hoeve, E. S., Kelly, G., Luz, S., Ghanshani, S., & Bhatnagar, S. (2013). Short-term and long-term effects of repeated social defeat during adolescence or adulthood in female rats. *Neuroscience, 249*, 63-73. doi: 10.1016/j.neuroscience.2013.01.073

Vidal, J., Bie, J., Granneman, R. A., Wallinga, A. E., Koolhaas, J. M., & Buwalda, B. (2007). Social stress during adolescence in Wistar rats induces social anxiety in adulthood

without affecting brain monoaminergic content and activity. *Physiol Behav., 92*(5), 824-830. doi: 10.1016/j.physbeh.2007.06.004

Vidal, J., Buwalda, B., & Koolhaas, J. M. (2011a). Differential long-term effects of social stress during adolescence on anxiety in Wistar and wild-type rats. *Behav.Processes, 87*(2), 176-182. doi: 10.1016/j.beproc.2011.03.004

Vidal, J., Buwalda, B., & Koolhaas, J. M. (2011b). Male Wistar rats are more susceptible to lasting social anxiety than Wild-type Groningen rats following social defeat stress during adolescence. *Behav.Processes, 88*(2), 76-80. doi: 10.1016/j.beproc.2011.08.005

Virgin, C. E., Jr., & Sapolsky, R. M. (1997). Styles of male social behavior and their endocrine correlates among low-ranking baboons. *Am.J.Primatol., 42*(1), 25-39. http://dx.doi.org/10.1002/(sici)1098-2345(1997)42:1<25::aid-ajp2>3.0.co;2-0

Von Frijtag, J. C., Reijmers, L. G., Van der Harst, J. E., Leus, I. E., Van den Bos, R., & Spruijt, B. M. (2000). Defeat followed by individual housing results in long-term impaired reward- and cognition-related behaviours in rats. *Behav.Brain Res., 117*(1-2), 137-146. http://dx.doi.org/10.1016/s0166-4328(00)00300-4

Vucetic, Z., Carlin, J. L., Totoki, K., & Reyes, T. M. (2012). Epigenetic dysregulation of the dopamine system in diet-induced obesity. *J.Neurochem., 120*(6), 891-898. doi:10.1111/j.1471-4159.2012.07649.x

Vyas, A., Mitra, R., Shankaranarayana Rao, B. S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *Journal of Neuroscience, 22*(15), 6810-6818. http://www.jneurosci.org/

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat.Protoc., 2*(2), 322-328. http://dx.doi.org/10.1038/nprot.2007.44

Walsh, R. N., & Cummins, R. A. (1976). The Open-Field Test: a critical review. *Psychol.Bull., 83*(3), 482-504. http://dx.doi.org/10.1037/0033-2909.83.3.482

Wang, J. L. (2006). Perceived work stress, imbalance between work and family/personal lives, and mental disorders. *Soc.Psychiatry Psychiatr.Epidemiol., 41*(7), 541-548. doi:10.1007/s00127-006-0058-y

Wardle, J., Steptoe, A., Oliver, G., & Lipsey, Z. (2000). Stress, dietary restraint and food intake. *J.Psychosom.Res, 48*(2), 195-202. http://dx.doi.org/10.1016/s0022-3999(00)00076-3

Warne, J. P. (2009). Shaping the stress response: interplay of palatable food choices, glucocorticoids, insulin and abdomincal obesity. *Molecular and Cellular Endocrinology, 300*, 137-146. http://dx.doi.org/10.1016/j.mce.2008.09.036

Watt, M. J., Burke, A. R., Renner, K. J., & Forster, G. L. (2009). Adolescent male rats exposedto social defeat exhibit altered anxiety behavior and limbic monoamines as adults. *Behavioral Neuroscience, 123*, 564-576. http://dx.doi.org/10.1037/a0015752

Weathington, J. M., Arnold, A. R., & Cooke, B. M. (2012). Juvenile social subjugation induces a sex-specific pattern of anxiety and depression-like behaviors in adult rats. *Horm.Behav., 61*(1), 91-99. doi: 10.1016/j.yhbeh.2011.10.008

Weaver, S. A., Diorio, J., Meaney, M. J., Weaver, S. A., Diorio, J., & Meaney, M. J. (2007). Maternal separation leads to persistent reductions in pain sensitivity in female rats. *Journal of Pain, 8*(12), 962-969. http://dx.doi.org/10.1016/j.jpain.2007.07.001

Weinstock, M. (1997). Does prenatal stress impair coping and regulation of hypothalamic-pituitary-adrenal axis? *Neuroscience & Biobehavioral Reviews, 21*(1), 1-10. http://dx.doi.org/10.1016/s0149-7634(96)00014-0

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W., Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *J.Clin.Invest, 112*(12), 1796-1808. doi:10.1172/JCI19246

Weiss, I. C., Feldon, J., & Domeney, A. M. (1999). Isolation rearing-induced disruption of prepulse inhibition: further evidence for fragility of the response. *Behavioural Pharmacology, 10*(2), 139-149. http://dx.doi.org/10.1097/00008877-199907000-00011

Weiss, I. C., Pryce, C. R., Jongen-Relo, A. L., Nanz-Bahr, N. I., & Feldon, J. (2004). Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. *Behav.Brain Res, 152*, 279-295. http://dx.doi.org/10.1016/j.bbr.2003.10.015

Whitaker, R. C., Wright, J. A., Pepe, M. S., Seidel, K. D., & Dietz, W. H. (1997). Predicting obesity in young adulthood from childhood and parental obesity. *New England Journal of Medicine, 337*(13), 869-873. http://dx.doi.org/10.1056/nejm199709253371301

Wilkin, M. M., Waters, P., McCormick, C. M., & Menard, J. L. (2012). Intermittent physical stress during early- and mid-adolescence differentially alters rats' anxiety- and depression-like behaviors in adulthood. *Behav.Neurosci., 126*(2), 344-360. doi:2012-04147-001 [pii];10.1037/a0027258

Willner, P. (1991). Animal models as simulations of depression. *Trends Pharmacol.Sci., 12*(4), 131-136. http://dx.doi.org/10.1016/0165-6147(91)90529-2

Willner, P. (1997). Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl), 134*(4), 319-329. http://dx.doi.org/10.1007/s002130050456

Willner, P. (2005). Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology, 52*(2), 90-110. doi:10.1159/000087097

Willner, P., Moreau, J. L., Nielsen, C. K., Papp, M., & Sluzewska, A. (1996). Decreased hedonic responsiveness following chronic mild stress is not secondary to loss of body weight. *Physiol Behav., 60*(1), 129-134. http://dx.doi.org/10.1016/0031-9384(95)02256-2

Willner, P., Muscat, R., & Papp, M. (1992a). An animal model of anhedonia. *Clinical Neuropharmacology, 15 Suppl 1 Pt A*, 550A-551A. http://dx.doi.org/10.1097/00002826-199201001-00286

Willner, P., Muscat, R., & Papp, M. (1992b). Chronic mild stress-induced anhedonia: A realistic animal model of depression. *Neuroscience & Biobehavioral Reviews, 16*, 525-534. http://dx.doi.org/10.1016/s0149-7634(05)80194-0

Willner, P., Towell, A., Sampson, D., Sophokleous, S., & Muscat, R. (1987). Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology (Berl), 93*(3), 358-364. http://dx.doi.org/10.1007/bf00187257

Winer, B. J. (1962). *Statistical Principles in Experimental Design*. New York, New York: McGraw-Hill.

Wolden-Hanson, T. (2010). Changes in body composition in response to challenges during aging in rats. *Interdiscip.Top.Gerontol., 37*, 64-83. doi: 10.1159/000319995

Wongwitdecha, N., & Marsden, C. A. (1996). Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. *Behav.Brain Res., 75*(1-2), 27-32. http://dx.doi.org/10.1016/0166-4328(96)00181-7

Wood, S. K., Walker, H. E., Valentino, R. J., & Bhathnagar, S. (2010). Individual differences in reactivity to social stress predict susceptibility and resilience to a depressive phenotype:

role of corticotrophin-releasing factor. *Neuroendocrinology, 151*, 1795-1805. http://dx.doi.org/10.1210/en.2009-1026

Wood, S. K., Baez, M. A., Bhatnagar, S., & Valentino, R. J. (2009). Social stress-induced bladder dysfunction: potential role of corticotropin-releasing factor. *Am.J.Physiol Regul.Integr.Comp Physiol, 296*(5), R1671-R1678. doi: 10.1152/ajpregu.91013.2008

Woods, S. C., & Seeley, R. J. (2000). Adiposity signals and the control of energy homeostasis. *Nutrition, 16*(10), 894-902. http://dx.doi.org/10.1016/s0899-9007(00)00454-8

Woods, S. C., Seeley, R. J., Rushing, P. A., D'Alessio, D., & Tso, P. (2003). A controlled high-fat diet induces an obese syndrome in rats. *J.Nutr., 133*(4), 1081-1087. http://jn.nutrition.org/

Wright, I. K., Ismail, H., Upton, N., & Marsden, C. A. (1991). Effect of isolation rearing on 5-HT agonist-induced responses in the rat. *Psychopharmacology (Berl), 105*(2), 259-263. http://dx.doi.org/10.1007/bf02244319

Wright, I. K., Upton, N., & Marsden, C. A. (1991). Resocialisation of isolation-reared rats does not alter their anxiogenic profile on the elevated X-maze model of anxiety. *Physiol Behav., 50*(6), 1129-1132. http://dx.doi.org/10.1016/0031-9384(91)90572-6

Xu, Y., Ku, B., Tie, L., Yao, H., Jiang, W., Ma, X. et al. (2006). Curcumin reverses the effects of chronic stress on behavior, the HPA axis, BDNF expression and phosphorylation of CREB. *Brain Research, 1122*(1), 56-64. doi:\10.1016/j.brainres.2006.09.009

Yau, J. L., Noble, J., & Seckl, J. R. (2001). Acute restraint stress increases 5-HT7 receptor mRNA expression in the rat hippocampus. *Neuroscience Letters, 309*(3), 141-144. http://dx.doi.org/10.1016/s0304-3940(01)02054-7

Yau, Y. H., & Potenza, M. N. (2013). Stress and eating behaviors. *Minerva Endocrinol., 38*(3), 255-267. http://www.minervamedica.it/en/journals/minerva-endocrinologica/

Yin, W., & Gore, A. C. (2010). The hypothalamic median eminence and its role in reproductive aging. *Ann.N.Y.Acad.Sci., 1204*, 113-122. doi: 10.1111/j.1749-6632.2010.05518.x

Yin, Y. Y., Ming, L., Zheng, L. F., Kan, H. W., Li, C. R., & Li, W. P. (2007). Bioactive compounds from Paecilomyces tenuipes regulating the function of the hypothalamo-hypophyseal system axis in chronic unpredictable stress rats. *Chin Med.J.(Engl.), 120*(12), 1088-1092. http://www.cmj.org/

Young, J. B. (2000). Effects of neonatal handling on sympathoadrenal activity and body composition in adult male rats. *Am.J.Physiol Regul.Integr.Comp Physiol, 279*(5), R1745-R1752. http://ajpregu.physiology.org/

Zeeni, N., Daher, C., Fromentin, G., Tome, D., Darcel, N., & Chaumontet, C. (2012). A cafeteria diet modifies the response to chronic variable stress in rats. *Stress*, 16(2), 211-219. http://dx.doi.org/10.3109/10253890.2012.708952

Zellner, D. A., Loaiza, S., Gonzalez, Z., Pita, J., Morales, J., Pecora, D. et al. (2006). Food selection changes under stress. *Physiol Behav., 87*(4), 789-793. http://dx.doi.org/10.1016/j.physbeh.2006.01.014

Zhang, W., Li, J., Zhu, J., Shi, Z., Wang, Y., & Kong, L. (2004). Chinese medicine Banxia-houpu decoction regulates c-fos expression in the brain regions in chronic mild stress model in rats. *Phytother.Res., 18*(3), 200-203. doi:10.1002/ptr.1391

Zhao, G., Ford, E. S., Dhingra, S., Li, C., Strine, T. W., & Mokdad, A. H. (2009). Depression and anxiety among US adults: associations with body mass index. *Int.J.Obes.(Lond), 33*(2), 257-266. doi: 10.1038/ijo.2008.268

Zheng, H., Lenard, N. R., Shin, A. C., & Berthoud, H. R. (2009). Appetite control and energy balance regulation in the modern world: reward-driven brain overrides repletion signals. *Int.J Obes.(Lond), 33 Suppl 2*, S8-13. http://dx.doi.org/10.1038/ijo.2009.65

Zheng, H., Liu, Y., Li, W., Yang, B., Chen, D., Wang, X. et al. (2006). Beneficial effects of exercise and its molecular mechanisms on depression in rats. *Behav.Brain Res., 168*(1), 47-55. doi: 10.1016/j.bbr.2005.10.007

Zurita, A., Martijena, I., Cuadra, G., Brandao, M. L., & Molina, V. (2000). Early exposure to chronic variable stress facilitates the occurrence of anhedonia and enhanced emotional reactions to novel stressors: reversal by naltrexone pretreatment. *Behav.Brain Res., 117*(1-2), 163-171. http://dx.doi.org/10.1016/s0166-4328(00)00302-8

Appendix A: Copyright Documentation

Permission from *PLoS ONE*

The following has been extracted from the *PLoS ONE* website (https://www.plos.org/open-access/license/): "PLOS applies the Creative Commons Attribution (CC BY) license to works we publish (read the human-readable summary or the full license legal code). Under this license, authors retain ownership of the copyright for their content, but allow anyone to download, reuse, reprint, modify, distribute and/or copy the content as long as the original authors and source are cited. No permission is required from the authors or the publishers."

Permission from Co-Authors

All co-authors the following cited manuscript provided permission to use all contents associated with the manuscript, as part of the present dissertation. Proof of permission is available upon request.

MacKay JC, James JS, Cayer C, Kent P, Anisman H, et al. (2014) Protracted Effects of Juvenile Stressor Exposure Are Mitigated by Access to Palatable Food. *PLoS ONE* 9(5): e96573. doi:10.1371/journal.pone.0096573