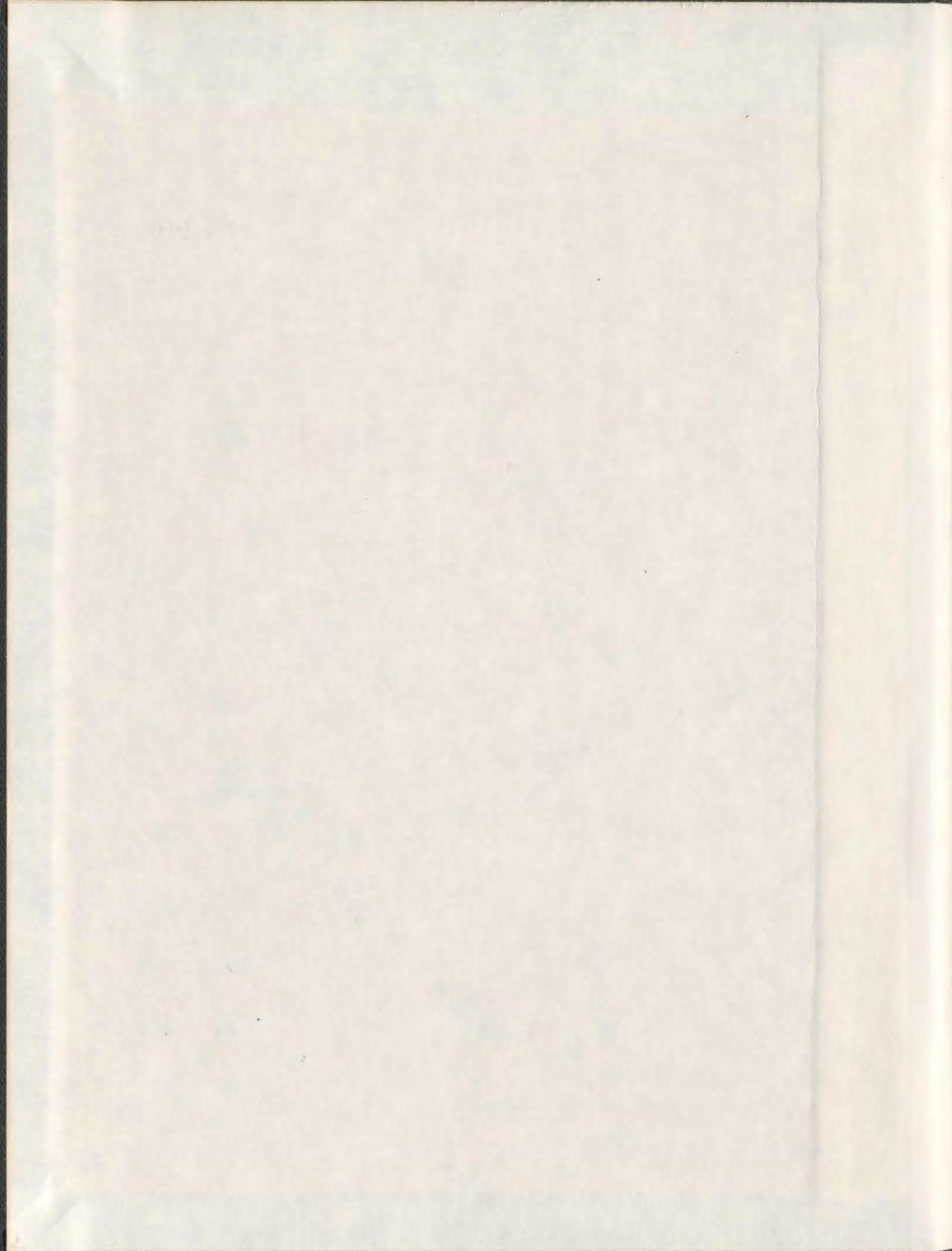
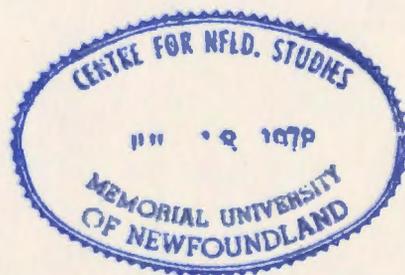


STRESS AND THE DEVELOPMENT OF DISORDERED
EATING IN RATS

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STRESS AND THE DEVELOPMENT OF DISORDERED EATING IN RATS

by

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Dedicated to Joanne Fry
Born June 4, 1978 and passed away November 9, 1997.

*In this short life
That only lasts an hour,
How much, how little,
Is within our power!*

~ Emily Dickinson

Abstract

Development of an eating disorder involves an interplay of factors, which may be environmental, biological, developmental, genetic, and/or psychological. All eating disorders are more prevalent in females, have an increased propensity for adolescent onset, and are, in most instances, precipitated by stressful life events. The value inherent in using an animal model to investigate the etiology of disordered eating lies in the real-life validity of such a model. Despite clinical evidence that early-life stress and heightened anxiety frequently precede the onset of pathological eating, animal models of eating disorders have not incorporated these findings to any great extent. Nor do most models take into account that the largest population of eating-disordered individuals is comprised of adolescent females and young women. The goals of this thesis were: (i) to present an overview of the physiological mechanisms that underlie stress-induced alterations in feeding systems and eating behaviours; and (ii) to examine potential factors that influence susceptibility to develop binge eating and anorexia using the most well-established animal models of these disorders. The research presented demonstrates that low levels of maternal care in early life are associated with greater vulnerability to the later development of stress-induced binge eating of highly-palatable food and, further, that this heightened vulnerability manifests in females during adolescence. In an activity-based animal model of anorexia nervosa (ABA), young adult animals that experienced early-life maternal separation lost weight faster, ate less, ran more, and required fewer days to reach removal criterion compared to their handled counterparts, with females, in particular, showing increased vulnerability. Finally, using a milder version of the ABA paradigm, early-life maternal separation increased females' susceptibility to ABA during adolescence, but not in adulthood, whereas males' susceptibility to ABA was increased only in adulthood. Together, these findings highlight the interplay between environmental, biological, and developmental factors in the etiology of binge eating and ABA.

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List of Abbreviations

5-HT: serotonin

α -MSH: alpha-melanocyte stimulating hormone

ABA: activity-based anorexia

ACTH: adrenocorticotrophic hormone

AFR: animal-facility reared

AgRP: agouti-gene-related protein

ANOVA: analysis of variance

BED: binge eating disorder

CART: cocaine- and amphetamine-regulated transcript

CCK: cholecystokinin

Cort: glucocorticoid

CRH: corticotropin-releasing hormone

CRH1: corticotropin-releasing hormone type 1 receptor

CRH2: corticotropin-releasing hormone type 2 receptor

DA: dopamine

Db: diabetes gene

DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th edition.

F: refeeding

GR: glucocorticoid receptor

H: handled

HP: highly-palatable

HPA: hypothalamic-pituitary-adrenal

KHz: kilohertz

LG: licking and grooming

LSD: least significant difference

M: mean

MANOVA: multivariate analysis of variance

MC3: melanocortin-3

MC4: melanocortin-4

mRNA: messenger ribonucleic acid

MS: maternally separated

NA: noradrenaline

NH: non-handled

NPY: neuropeptide Y

PND: postnatal day

POMC: proopiomelanocortin

PVN: paraventricular nucleus of the hypothalamus

R: restriction

r: correlation coefficient

SD: standard deviation

SE: standard error

SPSS: statistical package for the social sciences

ST: shock treatment

Co-authorship Statement

The chapters presented below comprise research that was designed and conducted by myself with the invaluable advice of a number of extremely supportive mentors. Chapter 1, *Introduction to Stress-Based Animal Models of Eating Disorders*, contains part of a book chapter that I wrote by invitation from Cella Olmstead (Queen's University). Cella provided helpful comments that guided my writing and suggested editing changes that improved the chapter's flow and comprehensibility. Virginia Grant (Memorial University) provided further editing suggestions that greatly improved the chapter's readability. This work is to be published, along with additional material, as "Animal Models of Eating Disorders" in the Neuromethods Series *Animal Models of Drug Addiction* (Cella Olmstead, Volume Editor). Chapter 2, *Variations in Maternal Care Influence Vulnerability to Stress-Induced Binge Eating in Female Rats*, was conducted under the supervision of Cella Olmstead and would not have been possible without the aid of Janet Menard (Queen's University) who provided the animals used in the study and, with a small team of undergraduate students, helped score rat maternal behaviours. The design of the study, care of the animals, experimental procedures and statistical analyses presented in the chapter, and the writing of the manuscript were completed by myself. Both Cella and Janet provided very helpful advice regarding editing of the manuscript which was published in the journal *Physiology and Behavior*. Chapter 3, *Early Maternal Separation Increases Symptoms of Activity-Based Anorexia in Male and Female Rats*, and Chapter 4, *Sexually Dimorphic Effects of Postnatal Treatment on the Development of Activity-Based Anorexia in Adolescent and Adult Rats*, were designed and conducted by myself, under the supervision of Virginia Grant. Patricia Barker (Vivarium, Memorial University) mated the dams as per our specifications and Malcolm Grant (Memorial University) provided very useful statistical advice. Manuscripts were prepared by myself, with the editing help of Virginia Grant. Chapter 3 has been accepted for publication in the *Journal of Experimental Psychology: Animal Behavior Processes* and Chapter 4 has been submitted for publication in *Developmental Psychobiology*. In the case of all four chapters, I am the principal author.

Chapter 1: Introduction to Stress-Based Animal Models of Eating Disorders

Animal models of eating disorders are not as well established as those of other diseases, such as drug addiction, diabetes, or stroke, for example, but the research in this area is progressing rapidly. The onset of an eating disorder entails an interplay between environmental and biological factors that predispose an individual to develop disordered eating, and possibly an increased propensity for anxiety and depression. The chance of recovery from an eating disorder is dismally low: more than 50% of patients in partial remission relapse within one year (Quadflieg & Fichter, 2003; Steinhausen, 2002). Isolating the causes and finding treatment options for eating disorders is difficult as no single animal paradigm can model the complexity of pathological eating, particularly as this is a large category that can be divided into several distinct disorders. The animal models presented below are those that best replicate the clinical features of anorexia nervosa and of binge eating, as a behavioural component of binge/purge anorexia, bulimia nervosa, and binge eating disorder. With the exception of obesity, which has yet to be recognized in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; American Psychiatric Association, 1994) as an eating disorder, anorexia, bulimia, and binge eating disorder encompass the majority of maladaptive eating behaviours in humans.

Anorexia nervosa is characterized by self-imposed weight loss to a body weight less than 85% of that expected for an individual's age and height. Anorexics exhibit increased activity levels and food obsessions, particularly related to the handling and preparing of food, but no significant disturbances in appetite (Garfinkel, 1974). That is,

anorexics avoid eating despite experiencing extreme hunger. The disorder may be separated into subtypes: restricting and binge eating/purging. Bulimia nervosa is sometimes considered a subtype of anorexia nervosa as both involve excessive preoccupation with food and body image, and bulimia is often preceded by a history of anorexia. The defining feature of bulimia is repeated episodes of overeating (i.e., bingeing) followed by compensatory behaviours such as vomiting, laxative use, or excessive exercise. Thus, the binge eating/purging subtype of anorexia nervosa overlaps considerably with bulimia nervosa, with the exception that anorexics are underweight whereas bulimics are typically of normal to low weight. Recurrent bouts of extreme overeating also occur in binge eating disorder, but with no subsequent compensatory behaviour.

Given that a primary feature of all eating disorders is disrupted feeding, any animal model must reproduce the maladaptive eating that is characteristic of that disorder. In the case of anorexia nervosa, food restriction should be self-imposed such that non-sated animals forego food consumption even when there is opportunity to eat. Despite the reduced intake of calories, anorexics exhibit high levels of activity, which should also be manifested in an animal model. Bulimia nervosa cannot be modeled fully in animals as there is no evidence that rodents or non-human primates initiate compensatory behaviours following binge eating episodes. By that criterion, gut emptying via gastric fistula does not model purging. In contrast, binge eating can be modeled effectively in animals if brief bouts of excessive food intake occur repeatedly over time, are enhanced by food palatability, but are independent of food deprivation and

circadian rhythms. To conform to the human disorder, food consumption of bingeing animals must be exaggerated compared to control animals. In addition to these fundamental changes in feeding behaviour, physiological and neuroendocrine changes accompany most eating disorders. A detailed analysis of these changes in anorexia nervosa suggests that some, but not all, are adaptations to reduced food intake and weight loss (Kaye, 2008). Bulimics and binge eaters exhibit distinct profiles of neuroendocrine changes although, in the absence of large longitudinal studies, it is impossible to determine which predates the other. Regardless of the causal relationship, the same changes should be evident in animal models of a particular disorder. Finally, any animal model should attempt to explain the higher rates of all eating disorders in females and the increased propensity for adolescent onset. In the following sections, animal models of anorexia nervosa and binge eating disorder are presented and evaluated based on how well they reproduce altered eating patterns and the accompanying physiological and neuroendocrine changes characteristic of each disorder.

1.1 Stress as a Precipitating Factor in Disordered Eating

Eating disorder patients exhibit increased anxiety and hyperresponsivity to stress. It is not surprising, therefore, that stressful life events often precipitate anorexic, binge eating, or bulimic episodes (Donohoe, 1984; Gluck, 2006; Kaye, 2008; Lo Sauro, Ravaldi, Cabras, Feravelli, & Ricca, 2008; Pyle, Mitchell, & Eckert, 1981; Swinbourne & Touyz, 2007; Wolff, Crosby, Roberts, & Wittrock, 2000). The effect of stress on food intake is moderated by stressor intensity in both humans and animals: mild or acute stress increases eating whereas severe stress decreases eating (Armario, 2006; Rowland &

Antelman, 1976; Stone & Brownell, 1994). When foods high in fat and sugar are available, stress increases intake of these “comfort” foods (Dallman, Pecoraro, et al., 2003; Dallman, Pecoraro, & la Fleur, 2005), even under conditions when reductions in eating are normally exhibited (Brown, Avena, & Hoebel, 2008). Given the well-established links between stress and eating disorders (Lo Sauro et al., 2008), a number of animal models have been developed that show altered food consumption and body weight following stress. In order to assess the validity of these animal models, it is important to understand the complex network of central and peripheral signaling peptides that control feeding, as well as the interactions between stress hormones and feeding peptides. The following overview will provide the necessary framework for understanding how stressful life events alter feeding behaviour in humans and other animals.

1.2 Peptide Control of Feeding

A schematic representation of peptidergic control of feeding is presented in Figure 1.1. Food intake and body weight are regulated primarily through neuropeptide production in the hypothalamus. The arcuate nucleus in this structure contains two groups of interconnected, ‘first order’ neurons that send axon terminals into the hypothalamic median eminence. The blood brain barrier is incomplete in the median eminence, allowing these neurons to contact the bloodstream (Peruzzo et al., 2000). One group of first order neurons produces anorexigenic (appetite-suppressing) peptides such as cocaine- and amphetamine-regulated transcript (CART) and the prohormone pro-opiomelanocortin (POMC). Cleavage products of POMC include the melanocortins adrenocorticotrophic hormone (ACTH) and alpha-melanocyte stimulating hormone (α -

MSH), the latter of which acts as an agonist at melanocortin-3 (MC3) and -4 (MC4) receptors to suppress food intake and weight gain (Cone, 2005). MC3 receptors are located primarily within the arcuate nucleus whereas MC4 receptors are found in many hypothalamic nuclei and widely distributed throughout the brain (Ellacott & Cone, 2006; Kishi et al., 2003). The second group of first order neurons acts in opposition to the first, releasing orexigenic (appetite-stimulating) peptides that include neuropeptide Y (NPY) and agouti-gene-related protein (AgRP). AgRP acts as an endogenous antagonist at MC3 and MC4 receptors (Cone, 2005; Ellacott & Cone, 2006), helping to maintain a balance between signals that increase and decrease food intake.

First order neurons in the arcuate nucleus send signals to 'second order' neurons in the dorsomedial, ventromedial, lateral, and paraventricular nuclei of the hypothalamus. Within second order nuclei, orexigenic and anorexigenic peptides are co-localized and co-released, activating receptors in overlapping regions. For instance, the lateral hypothalamus contains the orexigenic peptides melanin-concentrating hormone and orexin, as well as the anorexigenic peptide, CART. The paraventricular nucleus contains the anorexins cholecystokinin (CCK) and corticotropin-releasing hormone (CRH), but also orexigenic endogenous opioids. Whether orexigenic or anorexigenic peptides are released is largely dependent upon peripheral signals relating to the body's energy balance. One such signal comes from leptin, an anorexigenic peptide produced by adipocytes (fat cells) in direct relation to fat tissue mass. Leptin activates Ob receptors, the product of the diabetes (Db) gene (Chen et al., 1996), within the median eminence; it also crosses the blood brain barrier to activate Ob receptors located on orexigenic- and

anorexigenic-producing neurons in the arcuate, dorsomedial, ventromedial, lateral, and paraventricular nuclei. In a state of positive energy balance, this adiposity signal reaches the arcuate nucleus where POMC and CART neurons are stimulated and NPY and AgRP neurons are inhibited. In a negative energy balance, the opposite occurs. Ghrelin is another peripheral signaling peptide, produced by the stomach and, in smaller amounts, by the arcuate nucleus and pituitary (Date et al., 2000; Kojima et al., 1999). In contrast to leptin, ghrelin exerts orexigenic effects by stimulating NPY and AgRP expression in the hypothalamus (Kamegai et al., 2001; Wren et al., 2001) and by antagonizing the leptin pathway (Shintani et al., 2001). It is through these mechanisms that peripheral signals play an important role in regulating hypothalamic feeding-peptide function.

1.3 Stress Hormone – Feeding Peptide Interactions

Stress, defined as a physical or psychological event that causes a disruption in homeostasis (Reeder & Kramer, 2005), activates the hypothalamic-pituitary-adrenal (HPA) axis initiating a cascade-like secretion of hormones. The stress response is initiated in the paraventricular nucleus with the release of CRH. CRH stimulates high-affinity type 1 (CRH1) receptors located on anterior pituitary corticotrophs (Chen, Lewis, Perrin, & Vale, 1993), resulting in ACTH and α -MSH secretion as well as increased POMC synthesis and gene transcription (Lundblad & Roberts, 1988). Although ACTH and α -MSH suppress food intake, the anorexigenic effects of CRH are likely mediated directly through binding to low-affinity CRH type 2 (CRH2) receptors in the ventromedial (Hashimoto, Makino, Asaba, & Nishiyama, 2001) and paraventricular (Heinrichs & Richard, 1999) nuclei of the hypothalamus, as neither hypophysectomy nor

CRH1 receptor antagonists prevent CRH-induced anorexia in rats (Morley & Levine, 1982; Pellemounter et al., 2000). Stimulation of CRH2 receptors may be the mechanism through which leptin induces anorexic effects; leptin-stimulated release of CRH (Raber, Chen, Mucke, & Feng, 1997; Schwartz, Peskind, Raskind, Boyko, & Porte, 1996; Schwartz, Seeley, Campfield, Burn, & Baskin, 1996), upregulation of CRH2 receptor mRNA in the ventromedial hypothalamus of rats (Nishiyama, Makino, Asaba, & Hashimoto, 1999; Richard, Huang, & Timofeeva, 2000), and NPY inhibition (Cavagnini, Croci, Putignano, Petroni, & Invitti, 2000; Morley, Levine, Gosnell, Kneip, & Grace, 1987) all induce anorexic effects that can be prevented by CRH antagonists (Gardner, Rothwell, & Luheshi, 1998; Uehara, Shimizu, Ohtani, Sato, & Mori, 1998).

CRH-stimulated release of ACTH from pituitary corticotrophs activates melanocortin receptors in the adrenal cortex, resulting in glucocorticoid (Cort) secretion, namely cortisol in humans and corticosterone in rodents. Within the CNS, Cort binds to two types of receptors. The high-affinity mineralocorticoid receptor, located primarily in the hippocampus, is tonically activated and sensitive to the low Cort concentrations present under rest at the circadian trough (Spencer, Miller, Moday, Stein, & McEwen, 1993). Under nonstressful conditions, mineralocorticoid receptor occupation maintains low ACTH levels, keeping Cort concentrations at a minimum to preserve metabolic homeostasis (Dallman, Akana, Bhatnagar, Bell, & Strack, 2000). The low-affinity glucocorticoid receptor, widely distributed throughout the brain, is phasically activated by higher Cort concentrations which are present during the circadian peak preceding daily activity and, particularly, during periods of stress (Dallman et al., 2000; de Kloet,

Vreugdenhil, Oitzl, & Joëls, 1998; Reul & de Kloet, 1985). In response to stress, Cort binds to glucocorticoid receptors within the paraventricular nucleus, pituitary corticotrophs, and most notably the hippocampus, activating a negative feedback loop to reduce HPA-axis activity and halt further Cort secretion. This mechanism protects the brain from the detrimental effects of prolonged Cort exposure (McEwen & Stellar, 1993). Higher hippocampal glucocorticoid receptor density translates into greater sensitivity to the negative-feedback effects of circulating Cort and a faster “turning off” of the stress response (Bhatnagar & Meaney, 1995; Meaney, Aitken, Sharma, Viau, & Sarrieau, 1989). Glucocorticoid receptors are also found in high density in adipocytes, particularly intra-abdominal visceral fat, where their activation leads to lipid accumulation (Björntorp, 2001).

The HPA-axis response to stress is intensity-dependent with the magnitude of Cort release related to the perceived severity of the stressor. At low concentrations, Cort enhances body weight gain through facilitation of visceral fat deposition (Björntorp, 2001; Devenport, Knehans, Sundstrom, & Thomas, 1989), and promotes food intake via reductions in hypothalamic CRH and increases in hypothalamic NPY (Devenport & Torres, 1984; Devenport, Torres, & Murray, 1983; Schwartz, Strack, & Dallman, 1997; Strack, Sebastian, Schwartz, & Dallman, 1995; Tataranni et al., 1996; Tempel & Leibowitz, 1993; Zakrzewska et al., 1999). NPY-mediated increases in food intake and weight gain depend upon circulating Cort; NPY infusions fail to increase food intake and body weight in adrenalectomized rats unless these animals are co-treated with synthetic Cort (Sainsbury, Cusin, Rohner-Jeanrenaud, & Jeanrenaud, 1997). These orexigenic

effects are counteracted by a Cort-induced increase in Ob gene expression and plasma leptin levels (Dagogo-Jack, Selke, Melson, & Newcomer, 1997; Larsson & Ahrén, 1996; Spinedi & Gaillard, 1998). Leptin also acts at the adrenal level to dampen further Cort release (Bornstein, Uhlmann, Haidan, Ehrhart-Bornstein, & Scherbaum, 1997; Pralong et al., 1998). In the absence of Cort, the anorexigenic effect of leptin is maximized: leptin-induced decreases in body weight are greater and longer lasting in adrenalectomized animals and are dose-dependently reduced by exogenously-administered Cort (Zakrzewska, Cusin, Sainsbury, Rohner-Jeanrenaud, & Jeanrenaud, 1997). Adrenalectomy also alleviates obesity in leptin-deficient *ob/ob* and *db/db* mice (Makimura et al., 2000; Shimomura, Bray, & Lee, 1987).

In contrast to mineralocorticoid receptor occupancy at low concentrations, high Cort concentrations increase glucocorticoid receptor occupancy to produce dramatically different effects on feeding behaviour. High-dose Cort decreases body weight (Akana et al., 1999; Makino, Nishiyama, Asaba, Gold, & Hashimoto, 1998; Strack, Horsley, Sebastian, Akana, & Dallman, 1995), feeding efficiency (i.e., body weight gain per kcal of food intake; Devenport et al., 1989), and food intake (Makino et al., 1998), likely through increases in leptin secretion and upregulation of ventromedial hypothalamic CRH2 receptors (Makino et al., 1998). The weight reduction associated with glucocorticoid receptor stimulation is more pronounced in lean, than in fat, body mass. This results in an increased proportion of fat to lean tissue, particularly around the abdomen (Akana et al., 1999; Devenport et al., 1989; Pasquali & Vicennati, 2000), and may account for the higher proportion of visceral to subcutaneous fat mass in anorexics

(Zamboni et al., 1997). In sum, the modulation of feeding peptide systems by mild or severe stress provides a mechanism whereby stress may increase the propensity to develop an eating disorder.

1.4 Stress Manipulations in Animal Models of Eating Disorders

In animal models, mild stressors such as brief footshock (Strongman, 1965), mild tail pinch (Levine & Morley, 1981), bursts of noise (Rasbury & Shemberg, 1971), temporary restraint or handling (Badiani, Jakob, Rodaros, & Stewart, 1996), short-term social stress (Zelena, Haller, Halász, & Makara, 1999), saline injection (Booth & Campbell, 1975), and a small amount of wheel running (Lett, Grant, & Gaborko, 1996) all increase food intake and/or body weight. These acute stressors produce transient perturbations in the glucocorticoid circadian rhythm and only slight increases in daily mean Cort levels (Dallman et al., 2000). Chronic stressors of greater intensity have the opposite effect: prolonged footshock (Kuriyama & Shibasaki, 2004; Strongman, 1965), repeated tail pinch (Levine & Morley, 1981), sustained loud noise (Alario, Gamallo, Beato, & Trancho, 1987), extended immobilization (Harris, Palmondon, Leshin, Flatt, & Richard, 2006; Shimizu, Oomura, & Kai, 1989), 23 h/day social separation (van Leeuwen, Boone, Avraham, & Berry, 1997), long-term social stress (Zelena et al., 1999), and shifting animals from individual to paired housing (O'Connor & Eikelboom, 2000) all decrease food intake and/or body weight. These moderate stressors produce significant elevations in Cort so that mean daily levels are increased fivefold and glucocorticoid receptor occupation is chronic (Dallman et al., 2000). Given the differential effect on

food intake and body weight, acute stressors are commonly used to model bulimia and binge eating, whereas chronic stressors are incorporated into animal models of anorexia.

1.4.1 Binge Eating Models

Animal models of binge eating incorporate the phenomenon, well documented in humans, that stress-induced increases in feeding are magnified in the presence of calorie-laden snack foods (Oliver & Wardle, 1999). Consumption of high-fat, high-sugar “comfort” foods may occur because stress-induced opioid release suppresses HPA-axis activity (Dallman, Akana, et al., 2003). Intake of comfort foods sustains opioid release and increasingly inhibits HPA-axis activity (Kreek & Koob, 1998; Yeomans & Gray, 2002), creating a circle of events whereby humans and other animals may use comfort foods to “self-medicate” against stress (Dallman et al., 2005). High dietary restraint (Oliver & Wardle, 1999), which in women is correlated with hypercortisolemia (Anderson, Shapiro, Lundgren, Spataro, & Frye, 2002; McLean, Barr, & Prior, 2001; Rideout, Linden, & Barr, 2006; Rutters, Nieuwenhuizen, Lemmens, Born, & Westerterp-Plantenga, 2009), and Cort hyperreactivity (Epel, Lapidus, McEwen, & Brownell, 2001; Newman, O’Connor, & Conner, 2007), render individuals particularly susceptible to stress-induced bingeing. Eating comfort foods reduces the Cort response to stress (Markus, Panhuysen, Tuiten, & Koppeschaar, 2000) and provides short-term alleviation of negative emotions (Dubé, LeBel, & Lu, 2005). In rats, voluntary and periodic intake of food with high fat and high sugar content blunts HPA-axis reactivity by lowering hypothalamic CRH mRNA expression and reducing ACTH and Cort responses to stress (Bell et al., 2002; Kinzig, Hargrave, & Honors, 2008; la Fleur, Houshyar, Roy, &

Dallman, 2005; Pecoraro, Reyes, Gomez, Bhargava, & Dallman, 2004; Ulrich-Lai et al., 2007). In contrast, animals that only have access to a high-fat diet exhibit increases, rather than decreases, in HPA-axis activity (Kamara, Eskay, & Castonguay, 1998; Legendre & Harris, 2006; Tannenbaum et al., 1997). The high-fat-only diet appears to act as a nutritional stressor, highlighting the importance of intermittent and voluntary intake of high-fat food in stress reduction.

Corwin and colleagues developed an animal model of binge eating disorder based on clinical findings that human binge eating occurs in the absence of hunger (Marcus & Kalarchian, 2003), and is directed towards high-fat foods that are deemed “forbidden” and therefore self-restricted (Kales, 1990). In addition to unlimited chow, rats are given limited access (e.g., 2 hr) to high-fat vegetable shortening, multiple times per week for several weeks. Bingeing develops over time in that rats increase their intake of fat when it is available and restrict their intake of chow when fat is unavailable (Corwin et al., 1998; Dimitriou, Rice, & Corwin, 2000). This behaviour mimics the binge/compensation pattern typical of binge eating disorder and bulimia. Although bingeing on highly-palatable food may alleviate anxiety and reduce HPA-axis activity in the short-term, anxiety behaviours are increased in bingeing animals over the long-term (Cottone, Sabino, Steardo, & Zorrilla, 2008; de Araujo-Held, Martin, de Sousa Almeida, Luscher, & Corwin, 2002). This also characterizes bulimic and binge eating patients who experience increased anxiety following binges once the disorder is established.

A separate model of binge eating uses a restriction-refeeding/stress protocol to model dietary restriction and stress as precipitating factors in this disorder (Cattanach,

Malley, & Rodin, 1988; Polivy, 1996). Rats that experience repeated food restriction and refeeding, analogous to human “yo-yo dieting”, binge on high-fat, high-sugar food in the absence of hunger, long after restriction has ended (Hagan & Moss, 1997). When food restriction-refeeding cycles are followed by a mild footshock, bingeing on highly palatable food is initiated sooner and is more dramatic (Hagan et al., 2002). Bingeing is also more profound when cycles are introduced during the “adolescent” period in rats that experienced low levels of early-life maternal care (Hancock, Menard, & Olmstead, 2005), known to heighten HPA-axis reactivity to stress (Liu et al., 1997). Repeated fasting, stress, and bingeing on foods rich in sugar and/or fat decrease POMC and CRH2 receptor expression in the arcuate and ventromedial hypothalamus, respectively, increase NPY and Cort activity, and alter functioning of 5-HT, dopamine, and opioid systems in rats (Artiga et al., 2007; Avena, 2007; Boggiano et al., 2005; Brady, Smith, Gold, & Herkenham, 1990; Chandler-Laney, Castaneda, Pritchett, et al., 2007; Chandler-Laney, Castaneda, Viana, et al., 2007; Hagan & Moss, 1991; Makino et al., 1998; Makino, Asaba, Nishiyama, & Hashimoto, 1999; Sergeev et al., 2005). Disruptions in these systems are characteristic of both binge eating disorder and bulimia (Bailer & Kaye, 2003; Kaye, 2008; Kuikka et al., 2001), providing further face validity to the restriction-refeeding/stress model.

1.4.2 Activity-Based Anorexia Model

The activity-stress (Paré & Houser, 1973) or activity-based anorexia paradigm (Epling & Pierce, 1984, 1988; Epling, Pierce, & Stefan, 1983) is the most well-established animal model of anorexia. This protocol involves unlimited running-wheel

access (~ 22 to 23 hr daily), combined with limited food access (~ 1 to 2 hr daily). In both humans and rodents, running increases CRH, ACTH, and Cort secretion (Bi, Scott, Hyun, Ladenheim, & Moran, 2005; Burden, White, Dean, & Martin, 1993; Droste, Chandramohan, Hill, Linthorst, & Reul, 2007; Elias et al., 1991; Farrell, Garthwaite, & Gustafson, 1983; Fediuc, Campbell, & Riddell, 2006; Girard & Garland, 2002; Levin & Dunn-Meynell, 2004; Wong, Licinio, Gold, & Glowa, 1993), particularly when it is combined with food restriction (Broocks, Schweiger, & Pirke, 1990; Duclos, Bouchet, Vettier, & Richard, 2005). Early-life events that heighten HPA-axis reactivity to stress increase susceptibility to activity-based anorexia (Glavin & Paré, 1985; Hancock & Grant, in press) and are thought to precipitate the disorder in humans (Connan, Campbell, Katzman, Lightman, & Treasure, 2003). In the activity-based anorexia model, animals exhibit suppressed food intake during the once-daily meal, lower than normal body weight, and hyperactivity. These symptoms become more severe across days (Epling & Pierce, 1996; Kron, Katz, Gorzynski, & Weiner, 1978) and, in the absence of intervention, may lead to death (Routtenberg, 1968).

The activity-based anorexia model replicates many of the core behavioural and physiological characteristics of anorexia. For example, weight loss, food-intake suppression, and hyperactivity are more pronounced in young (Paré, 1975) and in female (Hancock & Grant, in press; Paré, Vincent, Isom, & Reeves, 1978) animals subjected to this protocol. Anorexics exhibit increased restlessness and excessive exercise that correlates with decreases in leptin levels (Holtkamp et al., 2006). In females, this leads to hypothalamic-gonadal dysfunction and amenorrhea (Baranowska, Baranowska-Bik, Bik,

& Martynska, 2008). In the activity-based anorexia model, estrous cyclicity is lost as activity levels increase (Pirke, Brooks, Wilckens, Marquard, & Schweiger, 1993) and leptin administration suppresses starvation-induced increases in wheel running (Exner et al., 2000). Wheel-running animals show increased hypothalamic serotonin (5-HT) (Avraham, Hao, Mendelson, & Berry, 2001) which, through stimulation of hypothalamic CRH release (Jones, Hillhouse, & Burden, 1976), has been proposed to mediate stress-induced anorexia (Morley & Levine, 1982). The role of 5-HT in anorexia nervosa is well documented (Kaye, 2008), providing further support for the validity of the activity-based anorexia model.

Finally, the activity-based anorexia model appears to mimic changes in cognitive function that develop with this disorder. Upon initial loss of 10-15% body weight, anorexia nervosa patients are cheerful, energetic, and mentally alert; with further weight loss, fatigue, irritability, and cognitive dysfunction ensue (Casper, 1998; Chui et al., 2008; Garfinkel & Kaplan, 1986; Steinglass, Walsh, & Stern, 2006). In animals, wheel running (Avraham et al., 2001) and moderate food restriction (Avraham, Bonne, & Berry, 1996) improve cognitive performance, as measured in a spatial-learning task, whereas extreme food deprivation leads to deficits in performance (Avraham, Bonne, & Berry, 1996). This suggests that, in addition to physiological and behavioural characteristics, the activity-based anorexia model may replicate some of the cognitive deficits displayed by anorexic patients following extreme weight loss.

1.5 Rationale for the Current Studies

Despite clinical evidence that early-life stress and heightened anxiety frequently precede the onset of disordered eating, animal models of eating disorders have generally not incorporated these findings. The value inherent in using an animal model to investigate the biological and environmental factors that influence pathological eating behaviours lies in the real-life validity of such a model. In the following three chapters, the most well-established animal models of binge eating disorder and anorexia nervosa are tested for their face validity or embodiment of the characteristics prevalent in the clinical population. Specifically, these models are used to examine the influence of stressful events in early life on the propensity for the later development of disordered eating. Further, studies investigating the mechanisms that underlie disordered eating most frequently use adult male animals despite the largest proportion of the clinical population of eating-disordered individuals consisting of adolescent females. In the following chapters, female rats are tested in all three sets of experiments and male rats are included for comparison in the latter two. In chapter 2, Hagan et al.'s (2002) animal model of binge eating disorder is used to investigate whether the early-life stress of low levels of maternal care increases susceptibility to footshock-induced binge eating of highly-palatable food in adolescent and adult female animals. Chapter 3 examines whether the early-life stress of maternal separation influences susceptibility of young adult male and female rats to the eating- and weight-suppressant effects of prolonged daily access to a running wheel, in combination with restricted feeding, in the activity-based anorexia paradigm. Finally, chapter 4 presents a modified version of the activity-based anorexia paradigm in which adolescent and adult, male and female rats from varying early-life

postnatal treatment groups are subjected to 2-h running wheel access, in combination with a restricted feeding schedule.

1.6 References

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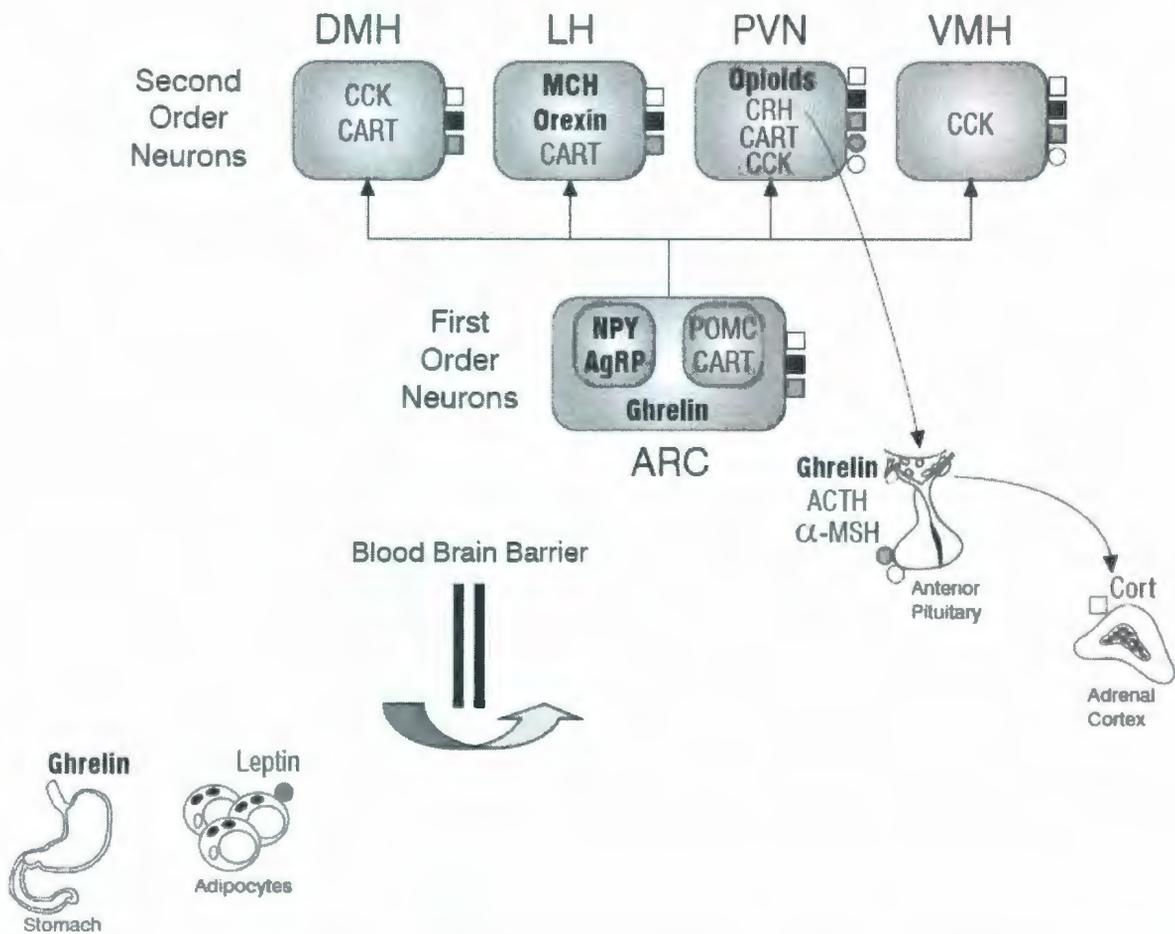


Figure 1.1. Schematic representation of peptidergic control of feeding. Information about the body's energy balance originates in peripheral organs, including the stomach and adipose tissue. Peptides, such as ghrelin and leptin, cross the blood brain barrier to convey signals to first-order neurons in the arcuate nucleus (ARC) of the hypothalamus. These project to second-order neurons in the dorsomedial nucleus (DMH), lateral nucleus (LH), paraventricular nucleus (PVN) and ventromedial nucleus (VMH) of the hypothalamus. Feeding-related neuropeptides within each hypothalamic nucleus are represented by thick (orexigenic) and thin (anorexigenic) fonts. □ melanocortin (MC) receptors; ■ leptin receptors; ▣ ghrelin receptors; ○ CRH receptors; ● glucocorticoid receptors. Refer to the text for information on neuropeptide function and interactions with the stress hormone system.

Chapter 2: Variations in Maternal Care Influence Vulnerability to Stress-Induced Binge Eating in Female Rats

2.1 Abstract

Binge eating disorder (BED) is characterized by intermittent, discrete periods of uncontrollable consumption during which large quantities of high-fat food are eaten. The onset of BED occurs most frequently in adolescent or young adult females and is often associated with a history of dieting and psychological stress. Animal research suggests the importance of two synergistic factors in the aetiology of binge eating: a history of restriction-refeeding cycles (i.e., “yo-yo” dieting) and exposure to acute stress. In the rat, natural variations in maternal licking and grooming (LG) of pups during the first week of life are associated with long-lasting individual differences in offspring sensitivity to stress. The current set of experiments examined the effects of restriction-refeeding-footshock cycles on intake of highly-palatable (HP) food in adolescent and adult female offspring of Low, Mid, and High LG dams. Following cycles of food restriction or unlimited food access, sated rats were exposed to footshock and their intake of HP food and chow was measured at 2, 4, and 22 h post-shock. Adolescent offspring of Low LG mothers displayed shock-induced binge eating, regardless of food-restriction history. In contrast, adolescent female offspring of Mid and High LG mothers failed to exhibit shock-induced increases in food intake. We saw no evidence of binge eating when shock was introduced in adulthood. These data suggest that low levels of maternal care in early life are associated with greater vulnerability to the later development of stress-related binge eating and further that this heightened vulnerability manifests during adolescence.

2.2 Introduction

Binge eating disorder (BED) is characterized in humans by recurrent episodes of uncontrollable eating during which large quantities of food are consumed within a discrete period (e.g., 2 h), without subsequent compensatory behaviours (i.e., laxative use or vomiting) to rid the body of excess calories (American Psychiatric Association, 1994). The quantity of food consumed during a binge is greater than that eaten by non-bingeing people during a comparable amount of time (American Psychiatric Association, 1994; Spitzer et al., 1992); the food is generally highly-palatable (HP), and dense in both calories and fat composition (Drewnowski, 1987; Drewnowski, Krahn, Demitrack, Naim, & Gosnell, 1992; Mitchell, Hatsukami, Eckert, & Pyle, 1985; Rosen, Leitenberg, Fisher, & Khazam, 1986; Spitzer et al., 1992). Consequently, most individuals diagnosed with BED are obese (Spitzer et al., 1992), and many have a history of dieting and extreme weight fluctuations (Spitzer et al., 1992, 1993). Although dieting has been associated with the onset of BED (Greeno & Wing, 1994; Howard & Porzelius, 1999; Polivy & Herman, 1985), a substantial number of binge eaters report that the onset of binge eating preceded, rather than followed, that of dieting (Abbott et al., 1998; Grilo & Masheb, 2000; Marcus, Moulton, & Greeno, 1995; Spitzer et al., 1993; Spurrell, Wilfley, Tanofsky, & Brownell, 1997). As well, stress plays an important role in the aetiology of binge eating (Michaud et al., 1990; Wolff, Crosby, Roberts, & Wittrock, 2000) and binge eaters rate laboratory-induced stressful situations as more intense than do non-bingeing individuals (Hansel & Wittrock, 1997). Furthermore, a history of dieting increases the risk for stress-induced hyperphagia in women given access to HP food (Frost,

Goolkasian, Ely, & Blanchard, 1982; Heatherton, Herman, & Polivy, 1991; Herman & Polivy, 1975; Schotte, Cools, & McNally, 1990), suggesting that food restriction and stress may act in synergy to precipitate BED (Hagan et al., 2002). Women are more likely than men to develop BED (Grunberg & Straub, 1992; Spitzer et al., 1993) and many binge eaters exhibit comorbid psychopathologies such as depression, substance abuse, panic disorder, and personality disorders (Yanovski, Nelson, Dubbert, & Spitzer, 1993). Recent evidence indicates that sexual and physical abuse during childhood, as well as bullying and discrimination by peers, are significant risk factors for adolescent development of BED (Striegel-Moore, Dohm, Pike, Wilfley, & Fairburn, 2002).

The development of an animal model is an important step for understanding the aetiology of BED, and for developing effective treatments. Most importantly, the utility of an animal model lies in its face validity or embodiment of the characteristics prevalent in the clinical population. In keeping with clinical observations, stress affects eating behaviour in laboratory animals. Acute psychological stressors such as tail pinch (Rowland & Antelman, 1976) and social defeat (Teskey, Kavaliers, & Hirst, 1984) can induce hyperphagia and lead to obesity. However the relationship between stress and eating is not straightforward. Cold swim (4°C) stress induces dramatic hyperphagic effects, particularly on intake of high-fat food (Vaswani, Tejwani, & Mousa, 1983), but exposure to a cold room (4°C) has no effect on chow consumption (Chaouloff, 1994). Electric shock produces hyperphagia in some instances (Ullman, 1952) whereas in others it has either inconsistent or no effects on intake (Robbins & Fray, 1980; Sterritt & Shemberg, 1963), or depends on shock duration, with short (3-s) shocks resulting in

hyperphagia and medium (30-s) and long (300-s) duration shocks resulting in hypophagia (Strongman, 1965).

A history of chronic food restriction and refeeding in female rats induces a greater magnitude of rebound hyperphagia compared to rats with a history of unlimited food access (Hagan & Moss, 1997). However, multiple cycles of restriction and refeeding, similar to human “yo-yo” dieting, are required before hyperphagia becomes apparent. For example, seven food restriction-refeeding cycles are insufficient to induce hyperphagia on HP food in sated rats (Hagan et al., 2002). Hagan and colleagues recently showed that the number of food-restriction and refeeding cycles required for inducing hyperphagia is substantially reduced when an acute stressor (i.e., footshock) is introduced at the end of each restriction-refeeding cycle (Hagan et al., 2002). In that study, female rats displayed hyperphagia on HP food following only three restriction-refeeding-shock cycles, whereas hyperphagia was absent in non-restricted, shocked rats. Hagan suggested that food restriction and acute stress act in synergy to induce hyperphagia in the rat (Hagan et al., 2002), thus mimicking reports of synergistic actions of dieting history and stress in the aetiology of binge eating in women (Frost et al., 1982; Heatherton et al., 1991; Herman & Polivy, 1975; Schotte et al., 1990).

Despite clinical evidence that traumatic events during childhood are strongly associated with binge eating onset (Striegel-Moore et al., 2002), these factors have not been incorporated into animal models of binge eating. Animal data support the importance of the early-life environment in the development of long-lasting individual differences in sensitivity to stress (Caldji et al., 1998; Champagne, Francis, Mar, &

Meaney, 2003; Liu et al., 1997; McIntosh, Anisman, & Merali, 1999; Ogawa et al., 1994; Penke et al., 2001; Wigger & Neumann, 1999). In the rat, one of the most reliable predictors of individual differences in sensitivity to stress is the degree of maternal care received in early life (Champagne et al., 2003; Liu et al., 1997). Thus, relative to adult rats that received high levels of maternal licking and grooming (LG) from their dams across the first week of life, adult offspring of Low LG mothers exhibit exacerbated physiological responses to stress, as measured by circulating stress hormones (Francis, Caldji, Champagne, Plotsky, & Meaney, 1999; Liu et al., 1997), and are behaviourally more fearful when tested in a variety of animal models of anxiety (Caldji et al., 1998; Francis, Caldji, et al., 1999).

Both endocrine responses to stress and eating behaviour are regulated by the hypothalamic-pituitary-adrenal (HPA) axis (Leibowitz, 1978; Leibowitz, Diaz, & Tempel, 1989; Leibowitz, Roossin, & Rosenn, 1984; Leibowitz, Sladek, Spencer, & Tempel, 1988; Plotsky, Cunningham, & Widmaier, 1989). Because early life events exert a long-lasting influence on HPA responsivity to stress (Penke et al., 2001) we might expect that these same events would similarly exert a long-lasting influence on eating behaviour. In fact, early maternal deprivation has been shown to increase rebound hyperphagia following cessation of a restricted-feeding schedule (Iwasaki, Inoue, Kiriike, & Hikiji, 2000), and to increase food intake and preference for HP food (McIntosh et al., 1999; Penke et al., 2001), especially in female rats (Iwasaki et al., 2000; McIntosh et al., 1999). However, it is not known whether natural variations of maternal care influence vulnerability to binge eating. Given the low prevalence of BED in the general population

(Spitzer et al., 1992, 1993), it is unlikely that dieting and stress, either alone or in synergy, elicit binge eating without some preexisting vulnerability. As such, the current study was designed to assess whether natural variations of maternal care influence shock-induced hyperphagia under conditions of food restriction or non-restriction, using the animal model of binge eating developed by Hagan and colleagues (2002).

Because BED is more prevalent in females (Spitzer et al., 1992) and the onset of binge eating generally occurs in adolescence or early adulthood (Binford, Mussell, Peterson, Crow, & Mitchell, 2004; Spurrell et al., 1997), the current study employed female rats with restriction-refeeding-shock cycles beginning in either adolescence (postnatal day [PND] 38) (Odell, 1990) or adulthood (PND 110). Given their relatively higher levels of sensitivity to stress, we predicted that offspring of Low LG mothers would display hyperphagia when exposed to either food restriction and footshock or footshock alone. Conversely, we expected that the higher levels of maternal care received by offspring of High LG mothers would serve as a protective factor, thus rendering them insensitive to the effects of shock on eating behaviour. The offspring of Mid LG mothers best represent the mean maternal LG frequencies of the normal population. Thus, we predicted that they would display patterns of hyperphagia induction similar to those obtained by Hagan and colleagues, using an unselected population of rats (Hagan et al., 2002). More specifically, we expected that offspring of Mid LG mothers would display hyperphagia when exposed to both food restriction and footshock, but not when exposed to footshock alone.

2.3 Method

2.3.1 Licking and Grooming Measures

For the first 7 days postpartum (PND 1 – PND 7), each of 24 adult Long-Evans dams was scored for maternal behaviour, once every 3 minutes during five daily 72-minute periods (Myers, Brunell, Shair, Squire, & Hofer, 1989). Two observations were performed during the dark cycle (at 6:00 a.m. and 8:00 p.m.) and three during the light cycle (at 10:00 a.m., 1:00 p.m., and 5:00 p.m.) for a total of 120 observations per mother per day. Maternal behaviour was classified according to the percentage of time spent: (a) licking or grooming any pup; (b) nursing in either an arched-back, blanket, or passive posture; or (c) not attending to pups. Previous studies have shown that frequencies of maternal licking and grooming of pups are the best predictor for individual differences in sensitivity to stress in the offspring (reviewed in Champagne et al., 2003). Thus, LG frequencies are the most commonly used measure for characterizing variations of maternal care. Because LG frequencies are normally distributed within a birth cohort (Champagne et al., 2003), at weaning (PND 21) we were able to classify the 24 litters into one of the following three groups: those of dams whose LG scores fell one standard deviation (*SD*) above (offspring of High LG mothers; $n = 24$), one *SD* below (offspring of Low LG mothers; $n = 43$), or within 0.23 *SD* of the cohort mean (offspring of Mid LG mothers; $n = 44$). Percentage of LG time within each group of High, Mid, or Low LG mothers was similar to that reported elsewhere (Champagne et al., 2003).

2.3.2 Subjects

At weaning (PND 21), female offspring of Low, Mid, and High LG mothers were assigned to one of two groups, according to the age at which the first restriction-

refeeding-shock cycle would begin: “adolescent” (N = 72) and “adult” (N = 39) groups experienced the start of restriction-refeeding-shock cycle 1 on PND 38 or PND 110, respectively. Behavioural testing was completed on PND 82 and PND 154 for these two groups. Adolescent and adult female offspring were acclimatized to a reverse 12-h light/dark cycle (lights on at 7:00 p.m.) and individual housing in opaque, bedded cages with free access to chow (Laboratory Rodent Diet 5001; LabDiet®; 4.0kcal/g, 59.8% carbohydrates, 4.5% fat, 23.4% protein) and water for 17 days prior to the start of restriction cycles. From PND 21 to PND 93, rats in the adult group were housed with a sibling. Adolescent offspring of Low, Mid, and High LG mothers and adult offspring of Low and Mid LG mothers were divided as equally as possible according to LG history into four weight-matched groups: a shocked, food-restricted group (adolescents: $n = 24$; adults: $n = 14$); a shocked, non-restricted group (adolescents: $n = 24$; adults: $n = 13$); a non-shocked, food-restricted group (adolescents: $n = 12$; adults: $n = 6$); and a non-shocked, non-restricted group (adolescents: $n = 12$; adults: $n = 6$). Group assignment remained constant throughout the study.

2.3.3 Restriction-Refeeding-Shock Cycles

At PND 38, food-restricted, adolescent rats assigned to the shocked and non-shocked groups began the first of four restriction days (R1-4) during which they were given 66% of the mean daily chow intake of non-restricted rats. At 10:00 a.m. daily, rats were weighed and returned to their home cages with a pre-measured amount of chow. The day subsequent to R4, all rats began six days of refeeding (F1-6) with unlimited access to chow and, on days F1-2, HP food (Hershey’s® Reese peanut-butter chips;

5.2kcal/g, 47% carbohydrates, 28% fat, 21% protein). Following F6, chow was removed from the home cage at 10:00 a.m. and, at 12:00 noon, food-restricted and non-restricted rats in the non-shocked groups were transported to a distant room containing shock-equipped operant chambers. Rats were placed individually in one of four identical polycarbonate operant chambers (24 cm × 20 cm × 30 cm) containing metal-rod flooring (4 mm wide separated by 1 cm) housed in a foam-lined sound-attenuating shell with ventilation fans to provide air circulation and to further mask extraneous noise. Illumination of a 4-watt light bulb exterior to the transparent chamber marked the beginning of a session, the duration of which was 87 s. The session ended with the extinguishing of the light. At the end of the session, non-shocked rats were returned to the colony room and immediately provided with pre-measured amounts of chow and peanut-butter chips. Food-restricted and non-restricted rats in the shocked groups were then transported to the operant chamber room and placed individually in one of the three remaining operant chambers. Illumination of the 4-watt light bulb exterior to the transparent chamber marked the beginning of a shock session during which four, 3-s bursts of 0.6 mA of electricity were delivered through the metal-rod flooring. This intensity of electric shock produced typical behavioural stress responses including urination, defecation, high-pitched vocalizations, and freezing but did not produce visible signs of tissue damage. Each shock was preceded, separated, and followed by 15 s of no shock for a total session duration of 87 s. The light was turned off at the end of the session. Footshock was chosen as a stressor in this study because its duration and intensity are easily controlled and standardized across subjects and because its onset and

offset are readily identified and allow for food-intake measures at discrete periods following stressor termination. Shocked rats were then returned to the colony and provided with pre-measured amounts of chow and peanut-butter chips. In maintaining the order of no-shock and shock sessions, the possibly stressful effects of odor and auditory cues associated with shock were precluded in the non-shocked groups. Chow and peanut-butter chip intake measures were performed at 2, 4, and 22 h post-shock, accounting for all spillage, after which the subsequent restriction-refeeding-shock cycle began. Naïve, adult rats experienced the same procedure with the exception that the first restriction-refeeding-shock cycle began at PND 110. Adolescent and adult rats underwent four consecutive restriction-refeeding-shock cycles.

We also wanted to determine whether shock-induced hyperphagia reflected a discrete response to acute stress rather than a pervasive, global increase of appetitive behaviour induced by repeated restriction-refeeding-shock cycles. Thus, we measured total chow intake, according to shock-treatment, during the 24-h period preceding shock-induced hyperphagia. Increases in chow intake 24 h prior to acute footshock would suggest that repeated restriction-refeeding-shock cycles have a pervasive effect on food intake, resulting in global increases of eating behaviour that manifest even in the absence of an acute stressor. Conversely, if chow intake did not differ in the 24-h period prior to shock, then this would suggest that shock-induced hyperphagia was specific to acute stress. Finally, because BED is characterized by recurrent episodes of overeating (American Psychiatric Association, 1994), only those rats exhibiting repeated bouts of

hyperphagia in response to footshock were considered to have developed stress-induced binge eating analogous to BED.

2.4 Results

2.4.1 Statistical Analyses

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 11.5 for Windows. To examine the effects of restriction-refeeding on body weight, a multivariate analysis of variance (MANOVA) was conducted using restriction treatment as the independent variable to compare weight at refeeding day one (F1) and on shock treatment day (ST) across all four restriction-refeeding-shock cycles. A Bonferroni adjustment was applied to all pairwise comparisons. Because shock treatment was hypothesized to differentially affect consumption according to history of maternal LG, planned orthogonal contrasts were performed to compare intake of HP food (peanut-butter chips) and chow according to shock treatment within LG history and restriction experience for adolescent and adult rats. The following orthogonal contrasts were performed for adolescent females in both the food-restricted and non-restricted groups: 1) shocked versus non-shocked offspring of Low LG mothers; 2) shocked versus non-shocked offspring of Mid LG mothers and 3) shocked versus non-shocked offspring of High LG mothers. The same set of contrasts was done for adult offspring of Low and Mid LG mothers in both the food-restricted and non-restricted groups. Because relatively few dams exhibited a level of maternal care significantly greater than the cohort mean, there were fewer offspring of High LG mothers and none remained for testing in the adult study. To verify that hyperphagia was

not a pervasive phenomenon but rather a discrete response to footshock, additional contrasts were used to compare total caloric intake during the 24 h prior to shock (F6) between shocked and non-shocked groups during those cycles in which shock-induced hyperphagia was present.

2.4.2 Effect of Restriction-Refeeding on Body Weight

As depicted in Figure 2.1, rats in the restricted groups weighed significantly less than non-restricted rats at the start of refeeding day one (F1) in each of the four food-restriction cycles in both the adolescent (p values $< .01$) and adult (p values $< .01$) groups. These group differences in body weight were no longer apparent following six days of refeeding. Thus, immediately prior to shock treatment, the weight of restricted and non-restricted rats did not differ both in the adolescent (p values $> .15$) and adult (p values $> .15$) groups.

2.4.3 Post-Shock Intake of Peanut-Butter Chips and Chow

2.4.3.1 Shocked vs. Non-Shocked Adolescent Offspring of Low LG Mothers: Food-Restricted Groups

Figure 2.2 illustrates discrete episodes of shock-induced hyperphagia across the restriction-refeeding-shock cycles in food-restricted, adolescent offspring of Low LG dams. For ease of presentation, only significant data are shown. During the first cycle, offspring of Low LG mothers in the shocked condition consumed significantly greater amounts of HP food, relative to offspring of Low LG mothers in the non-shocked condition. This shock-induced hyperphagia was evident at 2 h, $F(1,60) = 4.40$, $p < .05$, (Figure 2.2, bars 1 vs. 2), but not 4 h, $F(1,60) = 3.62$, $p = .06$, or 22 h, $F(1,60) = 0.73$, $p =$

.40, post-shock. There were no between-group differences in chow intake during cycle one (p values $> .24$), suggesting that the observed shock-induced hyperphagia was specific to increases in HP food intake, rather than global increases in appetitive behaviour. Cycle two was marked with the highest intensity and duration of shock-induced hyperphagia in food-restricted, adolescent offspring of Low LG mothers. At 2 h post-shock, shocked rats consumed significantly more HP food relative to non-shocked controls, $F(1,60) = 10.11, p < .01$, (Figure 2.2, bars 2 vs. 3). This effect was maintained at 4 h post-shock, $F(1,60) = 13.90, p < .01$, (Figure 2.2, bars 5 vs. 6) but had dissipated by the 22-h intake measure, $F(1,60) = 3.76, p = .06$). There were no group differences in chow consumption at the 2-h, $F(1,60) = 0.69, p = .69$, and 4-h, $F(1,60) = 0.48, p = .49$, post-shock intake measures within cycle two. During the third restriction-refeeding-shock cycle, food-restricted and shocked adolescent offspring of Low LG mothers consumed significantly more peanut-butter chips at 2 h post-shock compared to their non-shocked controls, $F(1,60) = 4.19, p < .05$ (Figure 2.2, bars 7 vs. 8). At the same time, shocked rats ate significantly less chow than did non-shocked controls, $F(1,60) = 8.88, p < .01$ (Figure 2.2, bars 7 vs. 8). There were no further differences in intake of peanut-butter chips at 4 h, $F(1,60) = 2.82, p = .10$, or 22 h, $F(1,60) = 3.01, p = .09$, post-shock. During the fourth cycle, food-restricted, adolescent offspring of Low LG mothers failed to demonstrate shock-induced hyperphagia at the 2-, 4-, or 22-h post-shock intake measures (p values $> .19$).

Adolescent offspring of Low LG dams in the shocked and non-shocked groups did not differ in their chow intake during the 24 h prior to any episode of shock-induced

hyperphagia (p values $> .34$). This supports the idea that footshock induces a discrete episode of binge-eating behaviour specific to acute stress, rather than reflecting pervasive increases in food intake following repeated restriction-refeeding-shock cycles.

2.4.3.2 Shocked vs. Non-Shocked Adolescent Offspring of Low LG Mothers: Non-Restricted Groups

Figure 2.3 portrays episodes of shock-induced hyperphagia in adolescent offspring of Low LG mothers in the non-restricted groups. For ease of presentation, only significant data are shown. In the first cycle, there were no post-shock group differences in intake of either HP food (p values $> .40$) or chow (p values $> .27$) at 2, 4, or 22 h post-shock. During cycle two, however, non-restricted adolescent offspring of Low LG mothers showed a relatively-intense, long-lasting shock-induced hyperphagia that was specific to HP food. At 2 h post-shock, non-restricted adolescent offspring of Low LG mothers consumed significantly more HP food in response to footshock relative to their non-shocked controls, $F(1,60) = 5.68, p < .05$ (Figure 2.3, bars 1 vs. 2). This effect was maintained at 4 h post-shock, $F(1,60) = 6.18, p < .05$ (Figure 2.3, bars 3 vs. 4) but had dissipated by the 22-h intake measure, $F(1,60) = 0.38, p = .54$. There were no group differences in chow consumption at the 2-h, $F(1,60) = 0.66, p = .42$, or 4-h, $F(1,60) = 0.27, p = .61$, post-shock intake measures within cycle two. During the third restriction-refeeding-shock cycle, there were no differences in intake of peanut-butter chips or chow at 2, 4, or 22 h post-shock (p values $> .12$). During the fourth cycle, non-restricted, adolescent offspring of Low LG mothers demonstrated shock-induced hyperphagia on HP food, $F(1,60) = 4.06, p < .05$ (Figure 2.3, bars 5 vs. 6) but not chow, $F(1,60) = 0.57, p$

= .45, at 4 h but not at 2 or 22 h post-shock (p values > .45). There were no group differences in chow intake during the 24 h prior to any episode of shock-induced hyperphagia (p values > .16), reflecting a discrete hyperphagic response to acute stress.

2.4.3.3 Adolescent Offspring of Mid and High LG Mothers: Food-Restricted and Non-Restricted Groups

Both food-restricted and non-restricted adolescent offspring of Mid and High LG mothers failed to display shock-induced hyperphagia across any of the four restriction-refeeding-shock cycles (p values > .08).

2.4.3.4 Adult Offspring of Low and Mid LG Mothers: Food-Restricted and Non-Restricted Groups

Adult offspring of Low and Mid LG mothers, in either food-restricted or non-restricted groups, showed no evidence of shock-induced hyperphagia in any of the four restriction-refeeding-shock cycles (p values > .07).

2.5 Discussion

The current data highlight the importance of the early-life environment in shaping individual vulnerabilities to stress-related binge eating. Adolescent offspring of Low LG mothers, in either the food-restricted or non-restricted groups, repeatedly displayed bouts of hyperphagia within the 22-h post-shock period. In contrast, this effect was not evident in adolescent offspring of either Mid or High LG mothers. We similarly saw no evidence of shock-induced hyperphagia when naïve offspring of either Low or Mid LG mothers were first exposed to restriction-refeeding-shock cycles in adulthood, suggesting that the

development of recurrent stress-induced binge eating is precipitated by anxiety-producing events during adolescence.

In both the food-restricted and non-restricted groups of adolescent offspring of Low LG mothers, shock-induced episodes of hyperphagia were specific to increases in intake of HP food (peanut butter chips), thus mirroring clinical reports that binge-eating bouts typically involve consumption of HP food that is both high-fat and dense in calories (Drewnowski, 1987; Drewnowski et al., 1992; Mitchell et al., 1985; Rosen et al., 1986; Spitzer et al., 1992). Importantly, these increases in HP food intake were noted at 2 and/or 4 h post-shock, but had dissipated by the 22-h post-shock measurement. This profile mimics that displayed by the clinical population in that binge eating typically occurs within a discrete time period of two to three hours (Spitzer et al., 1992).

Furthermore, the onset of shock-induced hyperphagia in both food-restricted and non-restricted adolescent offspring of Low LG mothers did not guarantee the occurrence of binge eating following each subsequent shock episode. In adolescent offspring of Low LG mothers, non-restricted rats exhibited hyperphagia following shock sessions two and four, but not shock session three, and food-restricted rats showed binge eating following shock sessions one through three, but not following shock session four. Interestingly, these findings are in line with BED-diagnosed individuals who report that they are better able to cope with stress and avoid binge-eating episodes on some occasions but not others (Wolff et al., 2000). Estrous cycle was not measured in the current study, however estrogen influences neurotransmitter system function, including that of serotonin which is known to exert both anorexigenic and antidepressant effects (Estrada-Camarena,

Fernandez-Guasti, & Lopez-Rubalcava, 2003, 2004; Galea, Wide, & Barr, 2001; Meguid et al., 2000; Rachman, Unnerstall, Pfaff, & Cohen, 1998). Further, estrogen influences HPA-axis functioning and physiological responses to stress (Isgor, Cecchi, Kabbaj, Akil, & Watson, 2003). Each restriction-refeeding-shock cycle spanned 11 days in the current study and, although speculative, it is possible that each shock-treatment day fell during a different stage of the 4-day estrous cycle (Butcher, Collins, & Fugo, 1974), perhaps influencing the occurrence of stress-induced binge eating. Finally, adolescent offspring of Low LG dams in the shocked and non-shocked groups did not differ in their chow intake during the 24 h prior to any episode of shock-induced hyperphagia. Thus, the episodes of shock-induced hyperphagia we observed in offspring of Low LG mothers seemed to reflect a discrete response to acute stress rather than a pervasive, global increase of appetitive responding consequent to repeated restriction-refeeding-shock cycles.

Despite these many similarities, the food-restricted and non-restricted adolescent offspring of Low LG mothers displayed differences in their shock-induced hyperphagic profiles. Specifically, shock-induced hyperphagia was apparent in food-restricted offspring in each of the first three restriction-refeeding-shock cycles, whereas non-restricted offspring required one additional restriction-refeeding-shock cycle before the onset of stress-induced hyperphagia. Non-restricted rats thereby displayed relatively fewer footshock-induced hyperphagic episodes. Also, the emergence of hyperphagia in non-restricted rats was typically delayed, occurring most often at 4 h post-shock. This differs from the more rapid onset of shock-induced hyperphagia consistently displayed at 2 h post-shock by food-restricted offspring of Low LG mothers. That stress-induced

binge eating appears more robust in restricted compared to non-restricted rats suggests that although a history of food restriction is not required for the onset of binge eating, its effects likely precipitate the disorder.

Food restriction activates the HPA axis causing an increase in circulating stress hormones such as corticosterone and adrenocorticotrophic hormone (ACTH) (El Fazaa, Gharbi, Kamoun, & Somody, 2000; Murphy & Wideman, 1992) in a manner similar to other stressors including social defeat (Pich et al., 1993), subordination (Blanchard, Sakai, McEwen, Weiss, & Blanchard, 1993), social isolation or crowding (Gamallo, Villanua, Trancho, & Fraile, 1986), restraint (Keim & Sigg, 1976), cold swim (Kioukia-Fougia et al., 2002), and noise stress (Michaud et al., 2003). Prior exposure to chronic stress has been shown to facilitate HPA-axis responsivity to a novel, acute stressor (Akana et al., 1996; Bhatnagar & Dallman, 1998; Scribner, Walker, Cascio, & Dallman, 1991). Thus, it seems possible that food-restriction-initiated changes at the level of the HPA axis might underlie the more robust binge eating we observed in restricted compared to non-restricted offspring of Low LG mothers. In support, a daily 2-h restricted-feeding schedule across 7 days resulted in rebound hyperphagia that was dramatically exacerbated in female rats exposed to space-restriction stress during refeeding (Inoue et al., 1998). Individually, prolonged cycles of food restriction or extreme acute stress might elicit binge eating as might a combination of various stressors, especially if responding to stress has been sensitized by low levels of early maternal care.

We were surprised that adolescent offspring of Mid LG mothers failed to display shock-induced hyperphagia when exposed to four restriction-refeeding-shock cycles.

These data stand in contrast to those of Hagan et al. (2002) in which an unselected population of adolescent, female rats exhibited shock-induced hyperphagia on HP food following three restriction-refeeding-shock cycles. To our knowledge, ours is the first study to explicitly use offspring of Mid LG mothers to investigate any behavioural effect of stress. We hypothesized that, although these offspring would be less vulnerable to stress-induced binge eating than those of Low LG mothers, they would nonetheless develop hyperphagia. That their eating behaviours were insensitive to the synergistic effects of food-restriction and footshock stress suggests that average levels of early-life maternal care do not induce vulnerabilities to stress-induced binge eating; such vulnerabilities result, not from a lack of high levels of maternal care, but from the sensitizing effects of low levels of maternal care during early life. As such, these data highlight the importance of accounting for early-life maternal care when investigating the behavioural effects of stress.

Our data also contradict those of Hagan et al. (2002) in that offspring of Low and Mid LG mothers failed to display shock-induced hyperphagia following four restriction-refeeding-shock cycles that began in adulthood. In contrast, offspring of Low LG mothers that were first exposed to restriction-refeeding-shock cycles in adolescence continued to exhibit shock-induced hyperphagia during restriction-refeeding-shock cycles 3 (in food-restricted rats) and 4 (in non-restricted rats), at which points these rats had matured into adulthood. The finding that binge-eating adolescent rats continued to exhibit shock-induced hyperphagia in adulthood whereas adult rats that remained naïve to stress during adolescence failed to exhibit shock-induced hyperphagia suggests that stress during

adolescence creates vulnerabilities for binge eating in adulthood. Furthermore, these findings highlight the importance of controlling and accounting for adolescent experiences of rats tested in adulthood, particularly in stress-related paradigms. Rats in the current study were bred and weaned in the stable conditions of our colony with no change in temperature, surroundings, or handlers. Our adult animals were thereby protected from some stressors that commonly occur during adolescence, such as being shipped to the research facility and the accompanying disruptions in light cycle, housing conditions, feeding cycle, and handlers.

Finally, our study differed from that of Hagan et al. (2002) on a number of variables, including rat strain (Long-Evans versus Sprague-Dawley), length of the food-restriction period in adult animals (four versus five days), type of HP food (peanut-butter chips versus Oreo® cookies), and light cycle in which footshock was applied (dark versus light). Any of these methodological differences could have contributed to our failure to observe shock-induced hyperphagia either in adolescent offspring of Mid LG mothers or in adult offspring of Mid or Low LG mothers first exposed to restriction-refeeding-shock cycles in adulthood. Nonetheless, at first glance it seems puzzling that adolescent, but not adult, offspring of Low LG mothers were highly sensitive to the effects of shock on binge eating. The impact of low levels of maternal LG on sensitivity to stress is well-documented in the literature, and is known to extend into adulthood. Thus, rats that receive relatively low levels of maternal LG in infancy are behaviourally more fearful (Caldji et al., 1998; Francis, Diorio, Liu, & Meaney, 1999; Liu et al., 1997; Menard, Champagne, & Meaney, 2004) and display exacerbated plasma ACTH and corticosterone

responses to acute stress in adulthood, compared to adult offspring of High LG mothers. Adult offspring of Low LG mothers also display decreased benzodiazepine receptor binding in the amygdala, higher corticotropin-releasing hormone (CRH) mRNA expression in the paraventricular nucleus of the hypothalamus (PVN), lower levels of hippocampal glucocorticoid receptor (GR) expression, and blunted negative feedback sensitivity to glucocorticoids, providing a mechanism for their heightened HPA responses to stress (Caldji et al., 1998; Liu et al., 1997; Meaney, 2001; Meaney, Aitken, Sharma, Viau, & Sarrieau, 1989). In summary, adult offspring of Low LG mothers exhibit increased vulnerability to stress and, once initiated, a relatively-prolonged stress response.

Despite this long-lasting influence of maternal care on stress responsivity, in the current study, footshock stress induced binge eating in offspring of Low LG mothers only when introduced during adolescence. This suggests that adolescence might be period of hypersensitivity to stress. By extension, the manifestation of some stress-related responses during this period would likely be facilitated by, and perhaps even require, pre-existing vulnerabilities to stress. Although speculative, such a pattern would account for why we saw stress-induced binge eating in adolescent offspring of Low (but not Mid or High) LG mothers. It is also consistent with our failure to obtain shock-induced bingeing on HP food in adult animals. Other research supports the possibility for heightened sensitivity to stress-related hyperphagia during adolescence. Female rats exposed to maternal separation during infancy displayed food-restriction-induced rebound hyperphagia that depended on the age at which food restriction began (Iwasaki et al.,

2000). Rebound hyperphagia was significantly greater in maternally-separated rats than in non-separated rats, but this difference was only apparent when food restriction began at 6 or 9, but not at 3 or 12, weeks of age (Iwasaki et al., 2000). In the current study, food restriction in adolescent rats began at 5.5 weeks whereas adult rats experienced the first cycle of food restriction at 15.5 weeks of age. These data support the concept of a period of adolescent hypersensitivity to stress and corroborate the lack of shock-induced binge eating in adult rats in the current study. At any rate, our data are consistent with clinical evidence that the onset of binge eating generally occurs in adolescence or early adulthood (Spurrell et al., 1997), and complement evidence of childhood adversity in BED-diagnosed individuals (Striegel-Moore et al., 2002).

The neural mechanisms underlying the hypersensitivity of offspring of Low LG mothers to shock-induced hyperphagia during adolescence are unknown. Adolescence is a period of continuing neurobiological development. For example, the noradrenergic system in the hypothalamus undergoes maturational changes throughout adolescence (Choi & Kellogg, 1996; Choi, Weisberg, & Kellogg, 1997). The hypothalamic noradrenergic system is heavily implicated in HPA axis regulation of endocrine responses to stress (Leibowitz et al., 1989; Plotsky et al., 1989), as well as the control of eating behaviour (Leibowitz, 1978; Leibowitz et al., 1984, 1988). Injecting noradrenaline (NA) into the PVN results in relatively-immediate increases in short-term feeding in sated rats (Bishop, Currie, & Coscina, 2000) and antagonizes the satiety-inducing effects of serotonin (Weiss, Papadakos, Knudson, & Leibowitz, 1986). In addition, injections of NA into the PVN increase extracellular dopamine (DA) in the nucleus accumbens

(Hajnal, Mark, Rada, Lenard, & Hoebel, 1997), thus augmenting the reinforcing value of food (Zhang, Balmadrid, & Kelley, 2003) and increasing appetitive behaviour (Hanlon, Baldo, Sadeghian, & Kelley, 2004; Swanson, Heath, Stratford, & Kelley, 1997).

Adversity in the early life may alter the normal developmental trajectory of the NA system in a manner that sensitizes NA responses to stress. In fact, maternal separation in infancy leads to relatively higher levels of restraint-stress-induced increases of NA release in the PVN (Liu, Caldji, Sharma, Plotsky, & Meaney, 2000). As noted by Choi and colleagues, systems undergoing change, such as the noradrenergic system in adolescence, are vulnerable (Choi et al., 1997), possibly rendering individuals already sensitized by early-life adversity at greater risk for adolescent onset of various disorders, such as BED.

In summary, the value inherent in using an animal model to investigate developmental changes or neural mechanisms that influence pathological behaviours, such as binge eating, lies in the real-life validity of such a model. The current data suggest that the acute effects of stress elicit binge eating and are exacerbated by a history of food restriction. In addition, our findings highlight the importance of early-life experience in precipitating binge-eating onset, thereby paralleling reports by BED patients of stressful events in childhood and adolescence. Inconsistencies in the literature regarding the effects of various psychological stressors on food intake could potentially be explained by individual differences in early-life experiences. That adolescent animals exhibited stress-induced binge eating that continued into adulthood whereas adults, naïve

to stress during adolescence, failed to demonstrate hyperphagic responses to stress, further validates this model.

2.6 References

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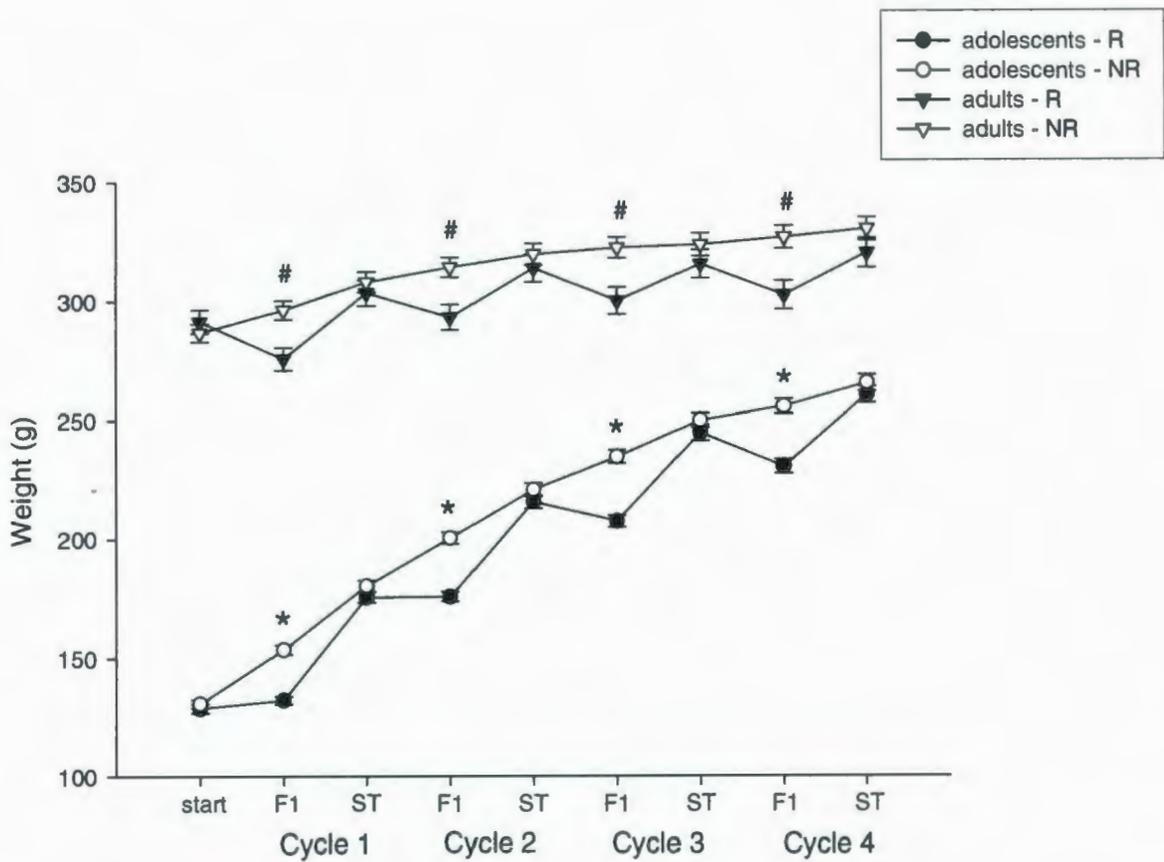


Figure 2.1. The effect of four restriction-refeeding cycles on body weight (g) of restricted (R) and non-restricted (NR) adolescent and adult rats. Across each of the four cycles, body weight at the start of refeeding day one (F1) was significantly lower in R compared to NR rats both in the adolescent and adult groups. Following six days of refeeding, R and NR rats did not differ in body weight on the morning of shock treatment (ST), both in the adolescent and adult groups. Data points represent group mean \pm SE; * $p < .0001$ and # $p < .005$ difference in weight between R and NR groups.

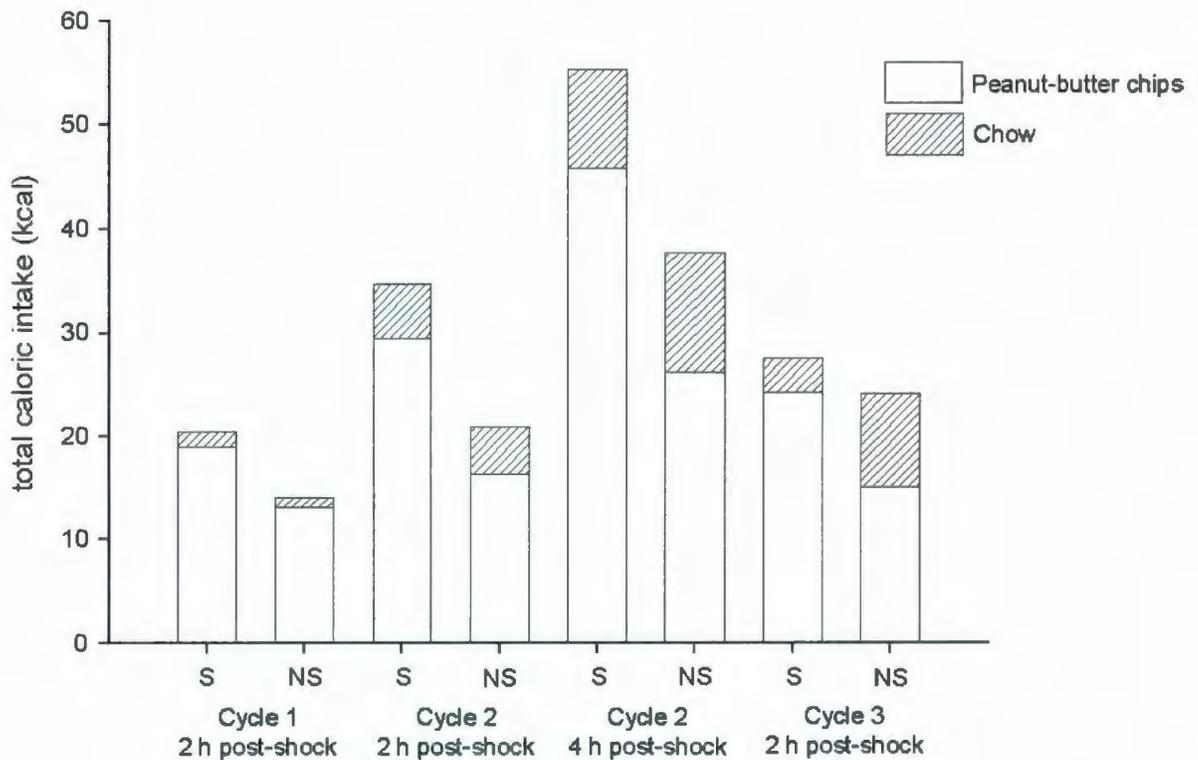


Figure 2.2. The hyperphagic effect of shock within food-restricted, adolescent offspring of low licking and grooming (LG) mothers across restriction-refeeding-shock cycles one through three. Peanut-butter chip intake (open bars) was greater in shocked (S) compared to non-shocked (NS) rats at 2 h post-shock across cycles one through three and at 4 h post-shock during cycle two (p values < .05). During cycle three, S rats consumed less chow (hatched bars) compared to NS controls (p < .01). Bars represent group means.

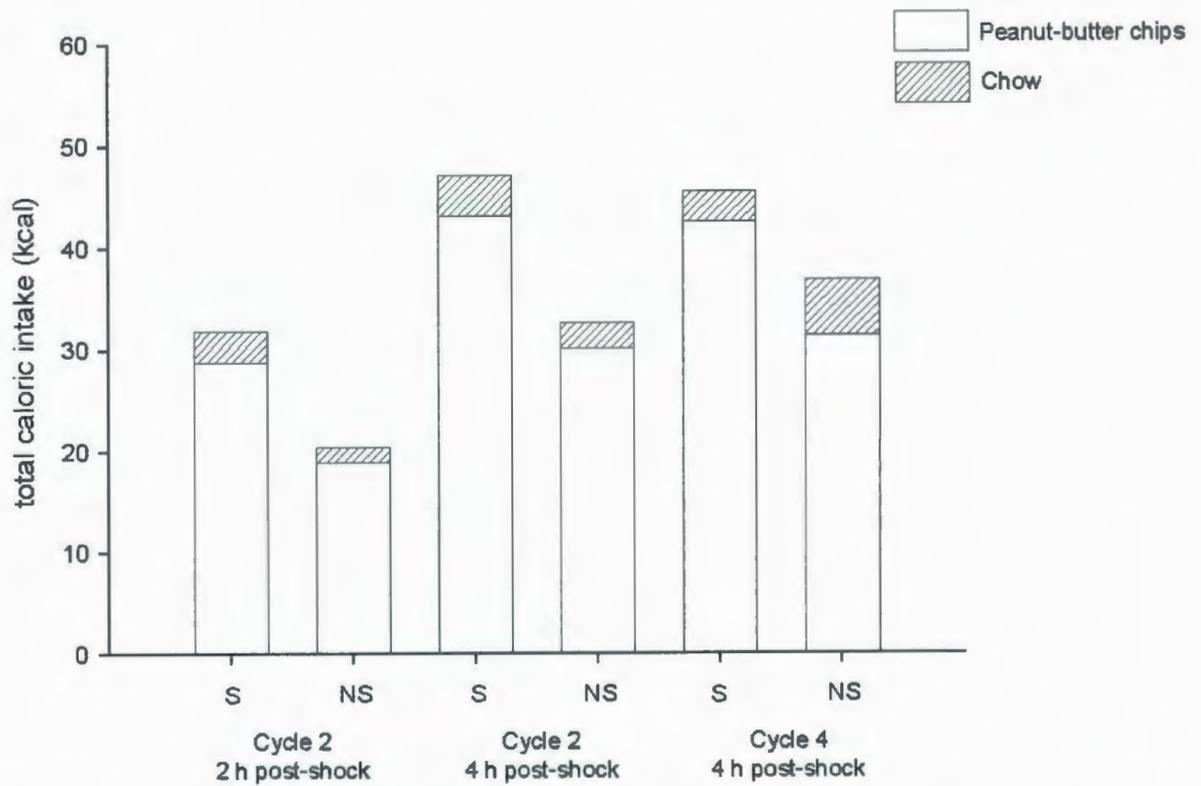


Figure 2.3. The hyperphagic effect of shock within non-restricted, adolescent offspring of low licking and grooming (LG) mothers across restriction-refeeding-shock cycles two and four. Peanut-butter chip intake (open bars) was greater in shocked (S) compared to non-shocked (NS) rats at 2 h post-shock in cycle two and at 4 h post-shock in cycles two and four (p values $< .05$). S and NS rats did not differ in chow (hatched bars) intake. Bars represent group means.

Chapter 3: Early Maternal Separation Increases Symptoms of Activity-Based Anorexia in Male and Female Rats

3.1 Abstract

Running activates the hypothalamic-pituitary-adrenal (HPA) axis, increasing the release of stress hormones known to exert anorexic effects. HPA-axis reactivity is strongly influenced by early postnatal manipulations, including removal of pups from the dam for short (“handling”) or prolonged (“maternal separation”) durations during the pre-weaning period. We examined the effects of handling and maternal separation on food intake, body-weight loss, and running rates of young adult male and female rats in the activity-based anorexia (ABA) paradigm. Postnatal treatment did not affect adaptation to a 1-hr restricted feeding schedule prior to the introduction of wheel running. During the ABA paradigm, maternally separated animals lost weight faster, ate less, ran more, and required fewer days to reach removal criterion compared to handled rats. Females were particularly vulnerable. These findings indicate that early postnatal treatment and sex influence ABA.

3.2 Introduction

Activity-based anorexia (ABA) is a syndrome that can occur in humans and other animals, characterized by suppressed food intake, below-normal body weight, and hyperactivity (Epling & Pierce, 1996; Kron, Katz, Gorzynski, & Weiner, 1978). In rats, ABA develops when animals are provided with a limited (~ 1 to 2 hr) daily period of food access and otherwise unlimited (~ 22 to 23 hr) access to a running wheel. Under these conditions, rats fail to consume enough calories during the once-daily meal to compensate for energy expended during wheel running. Counterintuitively, eating is reduced while body weight drops and running increases progressively across days, resulting in a “vicious cycle” (Dwyer & Boakes, 1997) that, without intervention, can lead to death by starvation (Routtenberg, 1968).

Running is a physiological stressor that increases metabolic activity and activates the body’s principal stress response system, the hypothalamic-pituitary-adrenal (HPA) axis (Tharp, 1975), initiating a cascade-like release of stress hormones. In both humans and rodents, voluntary running increases secretion of corticotropin-releasing hormone (CRH; Elias et al., 1991) and hypothalamic CRH mRNA expression (Bi, Scott, Hyun, Ladenheim, & Moran, 2005; Droste, Chandramohan, Hill, Linthorst, & Reul, 2007; Levin & Dunn-Meynell, 2004). Adrenocorticotropic-hormone (ACTH) release is augmented (Elias et al., 1991; Farrell, Garthwaite, & Gustafson, 1983; Wong, Licinio, Gold, & Glowa, 1993) with a corresponding increase in adrenal-gland weights (Burden, White, Dean, & Martin, 1993; Droste et al., 2007). Finally, plasma glucocorticoid levels (cortisol in humans, corticosterone in rodents) are higher post-running (Burden et al., 1993; Elias

et al., 1991; Farrell et al., 1983; Fediuc, Campbell, & Riddell, 2006; Girard & Garland, 2002; Wong et al., 1993) and correlate positively with exercise intensity (Farrell et al., 1983; Girard & Garland, 2002). In rats, when running is combined with food-restriction stress and resultant weight loss, as in the ABA paradigm, corticosterone release is exacerbated (Broocks, Schweiger, & Pirke, 1990; Duclos, Bouchet, Vettier, & Richard, 2005) and animals can develop activity-stress ulcers (Doerries, Stanley, & Aravich, 1991; Paré, Vincent, Isom, & Reeves, 1978a). There further exists a reciprocal relationship whereby running not only stimulates, but is maintained by, HPA-axis activity. Adrenalectomy essentially abolishes running behaviour (Leshner, 1971; Moberg & Clark, 1976; Richter, 1936) which can be dose-dependently restored by corticosterone replacement, with stress-level concentrations required for adrenalectomized animals to reach running levels comparable to those of sham-operated animals (Kendall, 1970; Leshner, 1971; Moberg & Clark, 1976). HPA-axis activity also modulates eating behaviour (Mastorakos & Zapanti, 2004). CRH exerts potent anorexic effects when injected intracerebroventricularly (Pellemounter et al., 2000; Richard, 1993; Rivest & Richard, 1990) or directly into the hypothalamic paraventricular nucleus (Krahn, Gosnell, Levine, & Morley, 1988). Administration of CRH antagonists eliminates the anorexic effects of exogenous CRH (Pellemounter et al., 2000), stress (Jochman, Newman, Kalin, & Bakshi, 2005), and wheel running by increasing meal size and reducing body-weight loss (Kawaguchi, Scott, Moran, & Bi, 2005; Rivest & Richard, 1990).

Given the relationships between HPA-axis activation, running, and eating, the possibility exists that animals with characteristically high or low stress reactivity may

exhibit varying levels of running activity and susceptibility to the effects of wheel running on eating and body-weight loss within the ABA paradigm. Early postnatal manipulations, specifically “handling” (Levine, 1975) and “maternal separation” (Lehmann & Feldon, 2000), are ethologically-relevant procedures (Calhoun, 1962) that affect the degree of maternal care pups receive. Maternal care, specifically licking and grooming of pups, epigenetically fine-tunes HPA-axis function during development (Meaney, 2001) and, consequently, affects offspring behavioural and physiological responses to stress. In most variations of the neonatal handling paradigm, rat pups are removed from their dam for a short period, usually 3 to 15 min, once daily across the first 14 or 21 postnatal days. Immediately following removal from the nest, pups are placed either individually or with littermates in a warm environment where they remain undisturbed until they are returned to their dam. Neonatal handling increases the average number, duration, and frequency (i.e., in KHz) of pup vocalizations (Bell, Nitschke, Gorry, & Zachman, 1971); mothers respond by retrieving the infants, returning them to the nest, and licking/grooming them (Bell et al., 1971). As such, handling significantly increases the frequency of licking and grooming of pups (Lee & Williams, 1974; Levine, 1975; Liu et al., 1997); it is this tactile stimulation that is believed to regulate pup physiology, affecting central nervous system development and neuroendocrine responses to stress (Caldji et al., 1998; Liu et al., 1997). Maternal separation procedures are similar to those of handling except that the duration of time spent away from the dam is significantly prolonged, usually 2 to 3 hr daily. If, during this prolonged separation period, maternal licking is simulated by stroking pups with a wet paintbrush or by

fostering them to another dam, the physiological and behavioural effects of maternal separation are reduced (Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001; Huot, Gonzalez, Ladd, Thirivikraman, & Plotsky, 2004).

Handling and maternal separation procedures during pre-weaning profoundly impact the neuroendocrinology, physiology, and behaviour of offspring during adolescence and adulthood. Compared to handled animals, maternally separated offspring exhibit increased hypothalamic CRH mRNA and greater median eminence CRH content under non-stress conditions (Plotsky & Meaney, 1993). In response to acute stress, maternally separated animals show a higher percentage of secretion and depletion of CRH storage pools as well as greater increases in peripheral corticosterone (Plotsky & Meaney, 1993). These effects persist throughout the lifetime (Meaney et al., 1996) and map onto stress-related behaviours with maternally separated animals exhibiting increased anxiety in a variety of measures of stress reactivity (Meaney et al., 1996).

Few studies have examined early-life environmental regulation of stress reactivity and later susceptibility to activity-based anorexia. Glavin and Paré (1985) found that precocious weaning, which heightens HPA-axis reactivity and anxiety behaviours in later life (Kikusui, Nakamura, & Mori, 2008), increased wheel running, mortality rates, and the development and severity of gastric ulcers in male rats subjected to the ABA paradigm. More recently, Carrera, Gutiérrez, and Boakes (2006) examined the effects of early postnatal handling of male and female rats on wheel-running rates, food intake, and weight loss in the ABA paradigm. Handling blunts stress reactivity (Meaney et al., 1996) and should thereby make rats less susceptible to the ABA-induced effects. Handling did

not, however, affect any of these measures in young adolescent males. In adult females, handled runners required more days to reach the removal criterion than did animal-facility reared (AFR) runners, but no between-group differences emerged in rate of weight loss, food intake, or wheel revolutions. That the early handling of animals, later subjected to the ABA paradigm, failed to produce increased resistance to the weight-loss and anorexic effects of prolonged wheel running is unexpected. However, the use of AFR animals as a control group in this experiment may have obscured evidence indicating the benefits of early handling (Levine, 2005; Pryce & Feldon, 2003). Throughout the pre-weaning period, AFR rats experienced routine cage changing comprised of disruption of mother and pups, exposure to a new homecage environment, and human tactile stimulation (Carrera et al., 2006). These manipulations increase maternal licking and grooming (Lee & Williams, 1974) and lead to adult stress-induced behaviours and neuroendocrine profiles comparable to animals that experience 15-min daily handling (Plotsky et al., 2005). As an alternative to animal-facility rearing, some researchers have used a non-handled (NH) control condition in which mother and pups experience a “radical absence of external stimulation” (Feldon & Weiner, 1992, p. 352) during the pre-weaning period, with no cage cleaning or human entry into the animal holding room. This procedure, however, creates a research caveat in that healthy pup development requires a minimal degree of stimulation stress (Levine, 1960), and the extreme deprivation inherent in the NH procedure results in adult deficits in emotionality and cognition (Levine, 1960) and has been used as an animal model of attention deficit and schizophrenia (Feldon & Weiner, 1992). In the current study, we compare handled and maternally separated

animals as the frequency of procedural manipulations is similar between groups, yet the short (handling) versus long (maternal separation) period of isolation from the dam leads to differential epigenetic modulation of HPA-axis reactivity so that “the effects of handling are almost completely the opposite of those of maternal separation” (Meaney et al., 1996, p. 57).

The present study examines the effects of prolonged periods of free wheel-running, in combination with a restricted feeding schedule, on food consumption, weight loss, and running rates in male and female animals that experienced handling or maternal separation across the first 14 postnatal days. Early research highlights the importance of the post-running feeding environment in contributing to the ABA effect; when the daily feeding period occurs in a familiar environment, food intake is significantly greater and weight loss significantly less than when feeding occurs in a novel environment (Routtenberg, 1968; Routtenberg & Kuznesof, 1967). In comparison to handled animals, maternally separated animals show greater novelty-induced hypophagia (Caldji, Francis, Sharma, Plotsky, & Meaney, 2000) and locomotor activity (Brake, Zhang, Diorio, Meaney, & Gratton, 2004). Therefore, animals in the current study were habituated to the procedures involved in the ABA paradigm prior to the onset of unlimited running to decrease any influence of novelty on wheel running rates, food intake, and weight loss.

In addition to running-wheel access, restricted feeding is an important procedural aspect of the ABA paradigm (Routtenberg, 1968; Routtenberg & Kuznesof, 1967); typically, wheel running and restricted feeding are concurrently introduced. Because food restriction activates the HPA axis (Chacón et al., 2005; Heiderstadt, McLaughlin, Wright,

Walker, & Gomez-Sanchez, 2000; Murphy & Wideman, 1992), preadaptation to a 1-hr/day restricted feeding schedule was implemented during a 13-day period, in the absence of unlimited wheel running. This would allow for the isolation of any effects of wheel running when introduced, and reveal any influence of postnatal treatment on rate of habituation or ability to learn a newly imposed restricted feeding schedule.

Furthermore, food intake measures during the ABA paradigm are conducted immediately following removal of rats from the restricted space and wire-mesh tactile environment of the running wheel. Both space confinement and wire-mesh flooring have anxiogenic properties and can affect food consumption (Inoue et al., 1998; Weiss, Feldon, & Domeney, 1999). To assess potential differences in the effects of these factors upon food intake of handled and maternally separated animals, rats were confined to small metal cages containing wire-mesh flooring for 2 hr prior to food intake measures during the preadaptation period. Finally, to control for the possible effects of genetics, genotypically similar pups were assigned to different postnatal treatment conditions (handled versus maternally separated; see Method for details).

3.3 Method

3.3.1 Subjects

3.3.1.1 Breeding

Virgin female and sexually experienced male Sprague-Dawley rats were mated such that pairs of sisters occupied a single cage during a seven-day breeding period and were impregnated by the same male. Three days following removal of the male from the females' cage, each pair of sisters was transported from the breeding facility at gestation

lengths ranging from approximately 6 to 7 days (estimated *post hoc* based on date of birth). There were two pairs of sisters; one pair was transported from the breeding facility 10 days after the first pair. On arrival, each of the four pregnant females was singly housed in an opaque polycarbonate birth cage (48 cm × 38 cm × 20 cm) lined with 4 cm of woodchip bedding (Sani-chips[®]; P.J. Murphy Forest Products, Montville, N.J., U.S.A.) and kept in a temperature-controlled ($22 \pm 1^\circ\text{C}$) holding room with a 12-hr light/dark cycle (lights on at 8:00 a.m.). Food (Prolab[®] Rat/Mouse/Hamster 3000 5P00; LabDiet[®]) and water were available *ad libitum*. Dams were left completely undisturbed with no moving of cages or changing of bedding throughout the remainder of pregnancy to avoid effects of stress on fetal development. Beginning 19 days following the first day of the breeding period, dams were checked daily for parturition at 9:00 a.m. and 5:00 p.m.; day of birth was designated post-natal day (PND) 0.

3.3.1.2 Postnatal Treatment

Two litters [sizes (male:female ratio): 18 (0.5) and 17 (1.4) offspring/litter], consisting of pups from one of each pair of sister dams, were designated “handled”. The remaining two litters [sizes (male:female ratio): 17 (0.6) and 19 (1.4) offspring/litter], consisting of pups from the alternate sister of each pair, were designated “maternally separated”. On PND 1, each dam was removed from the birth cage and individually placed in a clear polycarbonate holding cage (45 cm × 24 cm × 20 cm) lined with 2 cm of woodchip bedding and *ad libitum* access to food and water. Pups were subsequently removed from the birth cage by a gloved experimenter and each litter was individually placed in a plastic, paper-towel lined container measuring 26.5 cm × 18.5 cm × 11.0 cm

with 1.0 cm × 1.5 cm square openings spaced 0.75 cm apart along the four walls. Each container of pups was transported to an adjacent room, separated by a wall and closed door, and placed in an incubator (Nursery Hospital 1 Mechanical Brooder; internal measurements of 30 cm × 30 cm × 30 cm) maintained at 55-58% humidity and nest-like temperatures of 33-34° Celsius on PND 1 to 6, and 31-32° Celsius on PND 7 to 14 (Jans & Leon, 1983; Jans & Woodside, 1990). Litters designated “handled” remained apart from their dams for 15 min, from 9:45 a.m. to 10:00 a.m., on each of PND 1 through PND 14, inclusive. Litters designated “maternally separated” remained apart from their dams for 180 min, from 10:00 a.m. to 1:00 p.m., on each of PND 1 through PND 14, inclusive. Each litter of pups occupied one of four containers on each of the 14 days of handling or maternal separation with the container and its paper-towel lining remaining unchanged and unshared by any other litter.

Standardizing litter size and sex ratios is optimal as these factors affect adolescent and adult weights, food consumption, and anxiety-like behaviours (Dimitsantos, Escorihuela, Fuentes, Armario, & Nadal, 2007; Seitz, 1954). Therefore, on PND 1 during the period away from the dam, each litter was standardized to 12 pups: 6 males and 6 females. Also during this time, one-third of the woodchip bedding was removed from each birth cage and the remainder sifted with a kitty-litter scoop and replenished with fresh bedding. Following the 15 min (handled group) or 180 min (maternally separated group) period away from the dam, each litter of pups was immediately returned to its birth cage, each pup was individually rolled in bedding, and all pups were placed back into the area from which they had been retrieved by the experimenter. The dam was then

returned to the birth cage. On PND 14 during handling or maternal separation, two-thirds of the woodchip bedding was removed from each birth cage and replenished with fresh bedding. Litters were left undisturbed from PND 15 through 20, inclusive.

Pups were weaned on PND 21 and housed in groups of three same-sex littermates in clear polycarbonate holding cages (45 cm × 24 cm × 20 cm) lined with 2 cm of woodchip bedding and *ad libitum* access to food and water. On PND 32, animals were weighed, individually housed, and separated according to sex into two holding rooms where light cycle, temperature, and humidity levels were in accord with those of the original holding room. Twenty-four animals were randomly selected for use in the present study: six handled and six maternally separated males, and six handled and six maternally separated females (three of each sex from each of the four litters). Two female animals, one handled and one maternally separated, were removed prior to completion of the experiment and are not included in the analyses. The maternally separated female sustained a slight tail injury during habituation to the running wheel (see procedure) and failed to run thereafter. The handled female suddenly stopped eating early in the preadaptation period (see procedure) and was discovered to have loose top incisors.

3.3.2 Apparatus

There were 18 small metal cages and 18 running wheels. The small metal cages (18 cm × 25 cm × 18 cm) were used during periods of habituation and preadaptation to the feeding schedule (see procedure). The floor and front wall of the cages were constructed of wire mesh (0.2 cm wire spaced 1.1 cm apart) and the remaining three walls and lid of sheet metal. Water bottle holders were positioned on the front mesh wall

so that the spout of a water bottle could be extended to approximately 2 cm inside the cage. Running wheels, used during the ABA procedure, measured 11.5 cm in width with a circumference of 113.1 cm. The floor of each running wheel was constructed of wire mesh (0.2 cm wire spaced 1.1 cm apart) and the side walls of sheet metal. Attached to the edge of the rotating wall of the wheel was a small magnet that rotated during wheel revolutions. Extending from the opposite, stationary wall were two arms, spaced 180° apart, each equipped with a magnetic counter. A wheel revolution was recorded by a computer once the magnet had made a full 360° turn past both magnetic counters. Entry to and exit from the wheel was via an opening (11.7 cm × 7.5 cm) in the stationary wall across which a sliding metal door was placed during wheel running. The sliding metal door contained a hole through which the spout of a water bottle could be extended to approximately 2 cm inside the wheel. Feeding tests were conducted in clear polycarbonate feeding cages (45 cm × 24 cm × 20 cm), similar to the home cage except that the floor was left bare to allow for the recovery and weighing of uneaten food crumbs.

3.3.3 Procedure

3.3.3.1 Habituation

To reduce the impact of novelty-induced anxiety and resultant variations in locomotor activity and eating behaviours between groups, animals were habituated to the apparatuses and procedures associated with the ABA procedure. Beginning two days following individual housing (on PND 34), male and female handled and maternally separated animals began a total of eight consecutive habituation days during which they

were removed from the homecage and placed in a running wheel for 30 min and in a metal cage for 30 min. During each of the eight running-wheel exposures, wheel revolutions were recorded in six, 5-min intervals. Due to limited equipment, the order of running-wheel and metal-cage exposures was counterbalanced across animals; half of the males and females within each of the handled and maternally separated conditions experienced running-wheel exposure from approximately 9:30 a.m. to 10:00 a.m. and metal-cage exposure from 10:00 a.m. to 10:30 a.m., while the remaining half was exposed to the apparatuses during the same time period but in the opposite order. Animals were placed back into the homecages at 10:30 a.m. During habituation, animals continued to have free access to food and water in the homecage, but not in the running wheel or metal cage; daily food intake was not recorded during this period. Body weights were recorded immediately prior to the start of habituation day 5 (on PND 38) and after the habituation procedure had ended (on PND 42).

3.3.3.2 Preadaptation to a Restricted Feeding Schedule

Two days following the final habituation session (on PND 43), Baseline-1 body weights were recorded and all food was removed from the homecage at 12:00 noon; water access remained *ad libitum*. At approximately 10:00 a.m. on the following day, rats were removed from the homecages and weighed. For reasons explained in the introduction, each rat was then individually placed within a small metal cage with access to drinking water. At 12:00 noon, rats were removed from the metal cages and placed within one of 18 feeding cages. Approximately 30 g of the rats' regular food was placed in the feeder section of the wire cage lid and water was available *ad libitum*. Rats were

removed from the feeding cages at 1:00 p.m. and placed back into the homecage. Uneaten food was retrieved from the lid of the feeding cage, as were any food crumbs present on the bottom of the cage, and reweighed. This preadaptation procedure continued for a total of 13 days during which body weights were closely monitored to ensure that no rat fell below the removal criterion of 75% free-feeding body weight. At the end of the preadaptation period, rats were provided with free access to food and water within the homecage during a refeeding period of 6 days.

3.3.3.3 Resuming Restricted Feeding and Introducing the Activity-Based Anorexia Paradigm

Following the 6-day refeeding period (on PND 63), Baseline-2 body weights were recorded at 9:30 a.m. and all food was removed from the homecage. Body weights and 1-hr food intake were measured daily for 4 days following reintroduction of restricted feeding, according to procedures outlined in the preadaptation methodology. Following day-4 body-weight and food-intake measures, rats were removed from the feeding cages and placed back into the homecages until 11:30 a.m. at which time they were individually confined to a running wheel. Wheel revolutions were recorded in six, 220-min intervals across 22 hr of free wheel running. On each day of the ABA paradigm, rats were removed from the running wheels at 9:30 a.m., weighed, and individually placed within a feeding cage, as during the preadaptation period. At 10:30 a.m., rats were removed from the feeding cages and placed in the homecages. Uneaten food and crumbs were recovered from the feeding cages and weighed. At 11:30 a.m., rats were removed from the homecages and placed back into the running wheels for another 22-hr period of free

running. Rats were withdrawn from the ABA experiment when they reached the removal criterion, defined as a reduction in body weight to 75% that of Baseline-2 weight.

Experimental procedures adhered to the regulations set out by the Canadian Council on Animal Care and were approved and monitored by the Animal Care Committee.

3.4 Results

The following results present five indices commonly measured during habituation, preadaptation to a restricted feeding schedule, and the ABA paradigm. These include: baseline and percent-baseline body weights, daily food intake, wheel revolutions by interval and by day, days required to adapt to a restricted feeding schedule during preadaptation, and days required to reach the removal criterion during the ABA paradigm.

3.4.1 Body Weights

Weights were recorded two days prior to the start of habituation, at the beginning of habituation day 5, and one day subsequent to completing habituation sessions. Baseline-1 body weights were recorded on the day prior to the start of the preadaptation phase and were used as a measure against which percent body weight was compared during preadaptation. Baseline-2 body weights were recorded on the day prior to the reintroduction of restricted feeding and were used as a measure against which percent body weight was compared during reintroduction of restricted feeding and during the ABA paradigm. Figure 3.1 illustrates male and female handled and maternally separated rats' Baseline-1 and -2 body weights and percent Baseline weights throughout

preadaptation, restricted feeding, and ABA phases of the experiment (habituation data are not shown).

3.4.1.1 Habituation

A 2 (postnatal treatment) \times 2 (sex) \times 3 (day) repeated measures ANOVA, with day as the repeated factor, analyzed body weights prior to, at the mid-way point, and following habituation to running-wheel and metal-cage apparatuses in the absence of any food restriction. Weights increased across all three days, $F(2, 36) = 1788.28, p < .01$, more quickly in males than in females, $F(2, 36) = 87.74, p < .01$; male body weights were greater than those of females on all days, $F(1, 18) = 39.59, p < .01$. Body weights did not differ according to postnatal treatment, $F(1, 18) = 1.13, p = .30$, nor did postnatal treatment interact with day, $F(2, 36) = 1.64, p = .21$, or sex, $F < 1$. The three-way Postnatal Treatment \times Sex \times Day interaction was also non-significant, $F(2, 36) = 2.46, p = .10$.

3.4.1.2 Baseline 1

Baseline-1 body weights were recorded immediately prior to the start of preadaptation to a 1-hr daily feeding schedule. A 2 (postnatal treatment) \times 2 (sex) univariate analysis of variance (ANOVA) revealed no differences in body weight between handled and maternally separated animals during Baseline 1, $F(1, 18) = 1.39, p = .25$. Male body weights were significantly greater than those of females, $F(1, 18) = 73.33, p < .01$.

3.4.1.3 Preadaptation

A 2 (postnatal treatment) \times 2 (sex) \times 13 (day) repeated measures ANOVA, with day as the repeated factor, analyzed percent Baseline-1 body weights during the 13-day period of preadaptation to a 1-hr restricted feeding schedule. Body weight across days reflected a U-shaped function with initial reductions followed by a period of weight recovery, $F(12, 216) = 14.61, p < .01$. On average, female percent Baseline-1 body weights were significantly lower than those of males, $F(1, 18) = 9.61, p < .01$, with between-sex differences emerging across days, $F(12, 216) = 11.04, p < .01$. Body weights reached their lowest in males on preadaptation day 6, at which point they began to rebound; females continued to lose weight until preadaptation day 9. There were no differences in percent body-weight loss, overall or across days, between handled and maternally separated animals, and no Postnatal Treatment \times Sex or Postnatal Treatment \times Sex \times Day interactions, $F_s < 1$.

3.4.1.4 Baseline 2

Baseline-2 body weights were recorded at the end of the 6-day refeeding period. As during Baseline 1, a 2 (postnatal treatment) \times 2 (sex) univariate ANOVA revealed that handled and maternally separated animals were of similar body weights during Baseline-2 recordings, $F(1, 18) = 2.32, p = .15$, with males weighing more than females, $F(1, 18) = 133.17, p < .01$.

3.4.1.5 Restricted Feeding

A 2 (postnatal treatment) \times 2 (sex) \times 4 (day) repeated measures ANOVA, with day as the repeated factor, compared body weights, as a percent of Baseline-2 recordings, across the four days of restricted feeding that preceded the introduction of unlimited daily

wheel running. During this period, weights decreased linearly, $F(3, 54) = 26.69, p < .01$. There were no overall differences in percent Baseline-2 body weights between handled and maternally separated animals, $F < 1$, or between males and females, $F(1, 18) = 1.38, p = .26$. There was, however, a Postnatal Treatment \times Day interaction, $F(3, 54) = 6.78, p < .01$. Post-hoc analyses (Least Significant Difference; LSD) revealed that whereas handled animals showed significant weight decline across all four measurements (p values $< .05$), maternally separated animals' body weights dropped between day-1 and -2 measurements ($p < .01$) but stabilized thereafter (p values $> .50$). There were also sex-based differences in weight loss across the 4-day period, $F(3, 54) = 3.44, p < .05$, in that males' percent Baseline-2 weights dropped between days 1 and 2 of food restriction ($p < .01$) but stabilized thereafter (p values $> .10$) whereas female weights decreased steadily across all four measurement periods (p values $< .01$). There were no Postnatal Treatment \times Sex or Postnatal Treatment \times Sex \times Day interactions, $F_s < 1$.

3.4.1.6 Activity-Based Anorexia Paradigm

The ABA paradigm, in which 22 hr of daily wheel running was combined with the 1-hr restricted feeding schedule, began immediately following day-4 food restriction body-weight and food-intake measures. A 2 (postnatal treatment) \times 2 (sex) \times 5 (day) repeated measures ANOVA, with day as the repeated factor, was applied over the first 5 of 10 days of the ABA paradigm, during which none of the animals had reached the removal criterion of 75% Baseline-2 body weight. All animals lost weight during the first 5 days of the ABA paradigm, $F(4, 72) = 122.09, p < .01$. Although there was no main effect of postnatal treatment on percent Baseline-2 body weight, $F < 1$, there was a

significant Postnatal Treatment \times Day interaction, $F(4, 72) = 4.25, p < .01$, with handled and maternally separated animals differing in their rate of weight loss. On day 1 of the ABA paradigm, there was a 1.89% ($SE = 0.96$) mean difference in body weights with maternally separated animals slightly heavier than handled animals. By day 5 of the ABA paradigm, this trend had reversed to a 1.36% ($SE = 1.4$) difference in body weights with handled animals comprising the heavier group. Female percent Baseline-2 body weights continued to be significantly lower, on average, than those of males throughout the ABA paradigm, $F(1, 18) = 5.85, p < .05$. The three-way interaction was non-significant, $F(4, 72) = 1.62, p = .18$.

3.4.2 Food Intake

Figure 3.2 illustrates daily food intake of male and female handled and maternally separated rats throughout preadaptation to the 1-hr restricted feeding schedule, reintroduction of restricted feeding, and the ABA paradigm.

3.4.2.1 Preadaptation

A 2 (postnatal treatment) \times 2 (sex) \times 13 (day) repeated measures ANOVA, with day as the repeated factor, was used to analyze food intake during the preadaptation period. Food intake increased across the 13 days, $F(12, 216) = 235.71, p < .01$, with males consuming more food compared to females, $F(1, 18) = 19.05, p < .01$. There were no differences in food intake between handled and maternally separated animals during the 13-day preadaptation period, $F < 1$, and all two- and three-way interactions were non-significant (p values $> .05$).

3.4.2.2 Restricted Feeding

During reintroduction of restricted feeding that preceded the onset of wheel running, 1-hr food intake was measured daily for 4 days. A 2 (postnatal treatment) \times 2 (sex) \times 4 (day) repeated measures ANOVA, with day as the repeated factor, revealed an increase in food intake across all four measurements, $F(3, 54) = 64.18, p < .01$, with male food intake significantly greater than that of females, $F(1, 18) = 33.10, p < .01$. Handled and maternally separated animals consumed similar amounts of food during each day of the 4-day period, $F(1, 18) = 2.58, p = .13$. All two- and three-way interactions were non-significant (p values $> .25$).

3.4.2.3 Activity-Based Anorexia Paradigm

To determine whether wheel running produced an anorexic effect over and above the reductions in eating that resulted from restricted feeding, food intake during the 1-hr feeding period following the first 22 hr of wheel running (i.e., on ABA day 1) was compared to that of the 1-hr feeding period immediately preceding the onset of wheel running (i.e., at the day-4 restricted-feeding measurement). A 2 (postnatal treatment) \times 2 (sex) \times 2 (day) repeated measures ANOVA, with day as the repeated factor, revealed a significant effect of day, $F(1, 18) = 10.36, p < .01$, with less food intake following, as compared to preceding, the onset of wheel running. This analysis confirms that the introduction of wheel running produced the characteristic anorexic effect of a magnitude greater than that resulting from restricted feeding alone. There was no postnatal treatment effect and no significant two- or three-way interactions (p values $> .35$), suggesting that handled and maternally separated animals showed comparable reductions in food intake upon introduction of wheel running. As during restricted feeding in the absence of

running, males consumed significantly more food than did females, $F(1, 18) = 41.07, p < .01$.

Food intake over the first 5 of 10 ABA days, during which all animals remained in the experiment, was analyzed using a 2 (postnatal treatment) \times 2 (sex) \times 5 (day) repeated measures ANOVA, with day as the repeated factor. There emerged a main effect of postnatal treatment, $F(4, 72) = 6.07, p < .01$, with handled animals consuming significantly greater amounts of food, on average, compared to maternally separated animals. There was also a significant Postnatal Treatment \times Day interaction, $F(4, 72) = 8.31, p < .01$. Whereas maternally separated animals' food intake remained relatively stable across the first 5 days of the ABA paradigm (p values $> .11$), handled animals showed significant increases in food consumption. Finally, there were sex-based differences in food intake, $F(1, 18) = 48.32, p < .01$, with male intake greater than that of females throughout the 5 days of the ABA paradigm.

3.4.3 Wheel Running

3.4.3.1 Habituation

A 2 (postnatal treatment) \times 2 (sex) \times 2 (order of metal-cage, running-wheel exposure) \times 8 (day) \times 6 (interval) repeated measures ANOVA, with day and interval as repeated factors, analyzed running throughout the habituation period. Running rates were higher in females than in males, $F(1, 14) = 37.32, p < .01$, in handled than in maternally separated animals, $F(1, 14) = 14.29, p < .01$, and when metal-cage preceded running-wheel exposure, $F(1, 14) = 8.35, p < .05$. Across intervals, there was an overall decrease in running rates, $F(5, 70) = 24.71, p < .01$, and a Postnatal Treatment \times Interval

interaction, $F(5, 70) = 3.99$, $p < .01$, whereby handled animals ran significantly more than maternally separated animals during intervals 1 through 4 (p values $< .01$) but not 5 and 6 (p values $> .09$). Running rates increased across the habituation period, $F(7, 98) = 30.09$, $p < .01$, differing across days in a four-way interaction that further included postnatal treatment, sex, and order of metal-cage and running-wheel exposure, $F(7, 98) = 5.88$, $p < .01$. This interaction is illustrated in Figure 3.3. When removed from the homecage and placed directly in a running wheel, handled males ran significantly more than maternally separated males during habituation days 1 through 6 (p values $< .05$), whereas handled and maternally separated females ran at similar rates across the entire habituation period (p values $> .15$). When confined to a metal cage for 30 min prior to running-wheel exposure, maternally separated males slightly increased their running to a level similar to that of handled males (except on day 4, $p < .05$; all other p values $> .16$). In handled females, metal-cage confinement prior to running-wheel access reliably increased wheel running (p values $< .01$). In maternally separated females, prior metal-cage confinement resulted in a dramatic decrease in running rates during habituation days 1 through 3, and increased running during days 4 and 6 through 8 (p values $< .05$). This resulted in higher running rates in handled compared to maternally separated females on habituation days 1 through 4 (p values $< .05$), but similar rates on days 5 through 8 (p values $> .29$).

3.4.3.2 Activity-Based Anorexia Paradigm

Figure 3.4 illustrates male and female handled and maternally separated rats' running rates across the first 5 of 10 ABA days. The left-hand side of the figure portrays mean daily wheel revolutions per interval, while the right-hand side illustrates mean

wheel revolutions per day across six, 220-min intervals. A 2 (postnatal treatment) \times 2 (sex) \times 5 (day) \times 6 (interval) repeated measures ANOVA, with day and interval as repeated factors, revealed a significant main effect of sex, $F(1, 18) = 14.70, p < .01$, with female wheel-running rates higher than those of males. There was no overall effect of postnatal treatment on wheel revolutions, $F(1, 18) = 2.07, p = .17$. There was a significant increase across days in per-interval running rates, $F(4, 72) = 38.42, p < .01$, as illustrated in the left-hand portion of the graph. There was also a significant Postnatal Treatment \times Day interaction, $F(4, 72) = 2.52, p < .05$. Post-hoc analyses (LSD) revealed that, whereas maternally separated animals increased running rates across all days (p values $\leq .01$), handled animals ran at similar rates on days 1 and 2, and on days 3 and 4 (p values $> .07$). The right-hand portion of the graph illustrates a main effect of interval, $F(5, 90) = 23.51, p < .01$, with the lowest running rates occurring during interval 5 and the highest during interval 2. There was also a significant Postnatal Treatment \times Interval interaction, $F(5, 90) = 2.86, p < .05$, in that running during interval 2, which preceded dark onset, was significantly higher in maternally separated as compared to handled animals.

3.4.4 Adaptation to a Restricted Feeding Schedule

Adaptation to the 1-hr restricted feeding schedule was measured according to a survival criterion initially established by Routtenberg (1968) and since employed by others (e.g., Dwyer & Boakes, 1997; Lett, Grant, Smith, & Koh, 2001). An animal is considered to have successfully adapted to food restriction when its body weight on the fourth day of any consecutive 4-day period is equal to or greater than its weight on the

first day of that period. All animals successfully adapted to restricted feeding within the 13-day period. A 2 (postnatal treatment) \times 2 (sex) univariate ANOVA confirmed that there were no differences in the number of days required for handled ($M = 8.35$, $SE = 0.43$) and maternally separated ($M = 8.13$, $SE = 0.41$) animals to adapt to the 1-hr restricted feeding schedule during the preadaptation period, $F < 1$. Female rats required significantly longer ($M = 9.40$, $SE = 0.45$) than male rats ($M = 7.08$, $SE = 0.40$) to reach the adaptation criterion, $F(1, 18) = 15.13$, $p < .01$; there was no Postnatal Treatment \times Sex interaction, $F(1, 18) = 1.07$, $p = .31$.

3.4.5 Removal from Activity-Based Anorexia Paradigm

Removal from the 10-day ABA paradigm occurred when an animal's weight fell below 75% of Baseline-2 body weight. Two male rats (one handled, one maternally separated) failed to reach this removal criterion; number of days to removal for these rats was coded as 11. Number of days required to reach removal criterion was compared between male and female handled and maternally separated animals using a 2 (postnatal treatment) \times 2 (sex) univariate ANOVA. During ABA procedures, maternally separated animals reached 75% of Baseline-2 body weight in fewer days ($M = 6.82$, $SE = 0.47$) than did handled animals ($M = 8.53$, $SE = 0.46$), $F(1, 18) = 6.86$, $p < .05$. Also, female rats reached the removal criterion more quickly ($M = 6.60$, $SE = 0.42$) than did male rats ($M = 8.75$, $SE = 0.48$), $F(1, 18) = 10.76$, $p < .01$. There was no Postnatal Treatment \times Sex interaction, $F < 1$.

3.5 Discussion

The most significant finding of this study is that variations in postnatal treatment, known to exert long-lasting influence on behavioural and physiological responding to stress, alter individual vulnerability to the eating-suppressant and weight-reducing effects of wheel running in the ABA paradigm. In comparison to handled animals, maternally separated animals allowed 22-hr daily access to a running wheel, in combination with a 1-hr daily restricted feeding schedule, exhibited: (1) a faster rate of body-weight loss; (2) lower levels of food intake; (3) greater daily increases in wheel running; and (4) faster attainment of the removal criterion. Prior to the start of the ABA phase of the current study, all animals were habituated to the running wheels and preadapted to restricted feeding procedures. It is therefore unlikely that the effects of postnatal treatment on food intake, body-weight loss, and running behaviour during the ABA paradigm resulted from neophobia, to which maternally separated rats are particularly vulnerable (Caldji et al., 2000).

Reductions in food intake and body weight that occur upon introduction of ABA procedures are argued to reflect animals' inability to adapt to a concurrently imposed time-restricted feeding schedule rather than the effects of running, per se (e.g., Dwyer & Boakes, 1997; Kanarek & Collier, 1983; Paré et al., 1978a; Paré, Vincent, & Natelson, 1985; Routtenberg, 1968). However, Lett et al. (2001) demonstrated that, despite preadaptation to a restricted feeding schedule, rats subsequently given running-wheel access nonetheless demonstrated reductions in feeding and body weight that characterize ABA. The current study supports the findings of Lett et al.; upon introduction of wheel running, food intake dropped in all groups.

During the 13 days of preadaptation to a 1-hr restricted feeding schedule, food intake and body weights were monitored to determine whether adaptation differed as a function of postnatal treatment. Food intake increased across days to a similar extent in handled and maternally separated animals. At the same time, body weights dropped significantly upon introduction of restricted feeding but recovered within the 13-day period, resulting in a U-shaped pattern of weight loss and regain with no evidence of a postnatal treatment effect. Furthermore, all animals successfully adapted to the restricted feeding schedule with no difference in the number of days required by handled and maternally separated animals to reach the adaptation criterion. During the 4-day reintroduction of restricted feeding that immediately preceded the onset of unlimited wheel running, there was no effect of postnatal treatment on food intake. Again, restricted feeding led to a drop in body weight for all animals with maternally separated rats' body weights remaining stable after the day-2 measure. That implementation of a restricted-feeding schedule did not differentially influence food intake, and consequently body-weight loss, in handled and maternally separated animals is unexpected, given that food deprivation stress increases HPA-axis activity. These findings, however, are consistent with those of Murphy and Wideman (1992) wherein introduction of a 1-h restricted feeding schedule increased plasma corticosterone levels in Long Evans rats, more so than in stress-resistant Brattleboro rats, without differentially altering food intake between strains (Murphy & Wideman, 1992). Correlating neuroendocrine activity, such as plasma levels of ACTH and corticosterone or mineralocorticoid/glucocorticoid receptor occupancy, with behavioural measures of food intake and body-weight loss may help

clarify the seemingly anomalous lack of effect of postnatal treatment during food restriction in the current study, and identify the threshold at which stress influences eating behaviour. As there was no effect of postnatal treatment during the preadaptation or restricted-feeding periods, these data suggest that the increased rate of body-weight loss and reductions in food intake observed in maternally separated, compared to handled, animals during the ABA paradigm were a direct consequence of running-wheel access.

Across the first 5 days of the ABA paradigm, maternally separated animals exhibited a low, steady level of food intake and an additional average body-weight loss of 9.9% above the reduction seen during food restriction in the absence of wheel running. In comparison, handled animals showed an increase in food intake across those days and lost an average of 6.7% additional body weight. These differences in eating and weight loss corresponded with earlier attainment of the removal criterion in maternally separated animals. Although maternally separated animals showed greater increases in running across days than did handled animals, there were no within-day differences in wheel revolutions, nor did running rates differ during any of the four, 220-min intervals that preceded the feeding period. It is therefore unlikely that differences in post-running food intake and rate of body-weight loss within the ABA paradigm resulted from activity-induced differences in energy demand. Most likely, the differential effects of postnatal treatment on food intake and rate of body-weight loss during the ABA paradigm resulted from differences in HPA-axis reactivity. Running-induced suppression of food intake and body weight in rats corresponds with elevations in hypothalamic CRH mRNA expression (Kawaguchi et al., 2005) and increases in circulating ACTH (Wong et al., 1993) and

corticosterone (Burden et al., 1993; Wong et al., 1993). In maternally separated animals, these characteristics pre-exist (Plotsky & Meaney, 1993) and are likely to only be exacerbated by wheel running stress. As such, increased weight loss and decreased food intake in maternally separated animals subjected to the ABA paradigm may result from heightened release of stress hormones during running, which may account for the increased anorexic effect in these animals.

Females showed greater susceptibility to restricted-feeding and ABA procedures as demonstrated by more pronounced reductions in body weight and lower levels of food intake. These data accord with clinical studies documenting increased prevalence of anorexia in females (Lucas, Crowson, O'Fallon, & Melton, 1999), although the animal literature is less consistent (e.g., Boakes, Mills, & Single, 1999; Doerries et al., 1991; Paré, Vincent, Isom, & Reeves, 1978b). Increased vulnerability of females may be due to lower initial body weights, as lighter animals tend to lose weight more quickly within a restricted feeding regimen (Woods and Routtenberg, 1971; Yi & Stephan, 1996; as an exception, see Boakes et al., 1999). Alternatively, sex differences in the effects of food restriction and wheel running may result from differences in endocrine profile. HPA-axis reactivity, as measured by plasma levels of ACTH and/or corticosterone, is greater in females compared to males under resting conditions and following exposure to stressors including forced swim (Panagiotaropoulos et al., 2004; Wigger & Neumann, 1999), motorized wheel-running, immobilization, footshock (Kant et al., 1983) and the chronic mild stress paradigm (Dalla et al., 2005). Heightened HPA-axis reactivity may have led females in the current study to be more affected by the stress of a restricted feeding

regimen (Heiderstadt et al., 2000; Kiss, Jezova, & Aguilera, 1994) which would have been further exacerbated by wheel-running stress.

Differences in HPA-axis reactivity may have also led to divergent running profiles in handled and maternally separated animals. During the 8-day habituation period, running rates were higher in handled than in maternally separated animals, both overall and during the first 20 min of the 30-min running period, suggesting that the novelty suppressed activity levels to a greater extent in maternally separated than in handled animals. When 30 min of metal-cage confinement preceded running-wheel exposure, maternally separated males increased running to a level similar to that of handled males. Maternally separated females responded to metal-cage confinement with behaviour analogous to freezing, exhibiting extremely low levels of running during the first 3 days of habituation. By day 5, running rates had increased dramatically in maternally separated females so that they reached levels similar to those of handled females. Habituation results emphasize the importance of acclimatizing animals to running-wheel and metal-cage apparatuses, prior to start of experimental procedures, so that the confounding effects of novelty stress, which vary between postnatal treatment groups, can be eliminated.

Although there were no within-day differences in running between postnatal treatment groups during the first 5 days of the ABA paradigm, maternally separated animals' running increased across days to a greater extent than did that of handled animals. Studies show a correlation between HPA-axis activity and propensity for wheel running; mice selectively bred for high levels of running exhibit significantly higher

baseline corticosterone levels compared to control mice (Girard & Garland, 2002; Malisch et al., 2007). Increases in running rates are associated with the end of the light and start of the dark cycle (Eikelboom & Mills, 1988) with especially dramatic increases in selectively-bred runners (Girard & Garland, 2002). In the current study, the highest rates of running occurred during the 3:10 - 6:50 p.m. interval. This interval preceded onset of the dark cycle and encompassed the time point at which circadian-modulated levels of corticosterone are at their highest (Droste et al., 2003; Montano, Wang, Even, & Vom Saal, 1991). Furthermore, running during this interval was significantly higher in maternally separated animals, providing additional behavioural evidence that HPA-axis reactivity was increased in these animals, as compared to their handled counterparts.

Finally, the utility of an animal model lies in its embodiment of characteristics prevalent in the clinical population. The ABA paradigm is considered by many to be an animal model of anorexia nervosa (e.g., Epling & Pierce, 1996) with a high degree of face validity (Casper, Sullivan, & Tecott, 2008). Anorexia nervosa is an extreme condition with an estimated population prevalence of less than 1% (Kjelsås, Bjørnstrøm, & Götestam, 2004). Some predisposing factor, whether it be genetic, physiological, environmental, or a combination thereof, contributes to the development of anorexia nervosa. The current study indicates that, within this animal model of anorexia nervosa, increased susceptibility to ABA results from early-life events that lead to heightened stress reactivity in later life. HPA-axis hyperactivity, characteristic of maternally separated rats, is a neurohormonal marker of anorexia nervosa in humans (Gold et al., 1986; Hotta et al., 1986; Licinio, Wong, & Gold, 1996). It is unclear whether altered

HPA-axis function is a predisposing factor for anorexia or whether it is the result of suppressed eating and hyperactivity that characterize the condition (Hebebrand, Casper, Treasure, & Schweiger, 2004). It is worth noting in relation to the current findings that, on standardized measures of early life experience such as the Parental Bonding Instrument, anorexic patients report having experienced lower maternal “caring” (Gomez, 1984; Romans, Gendall, Martin, & Mullen, 2001) and more “inadequate” parental bonding during childhood (Di Pentima et al., 1998). Furthermore, prognosis for recovery from anorexia is directly related to perceived levels of parental bonding, with chronically ill patients indicating lower levels of maternal and paternal care as compared to patients partially or fully recovered from anorexia (Bulik, Sullivan, Fear, & Pickering, 2000). Low levels of parental bonding have been correlated with neurophysiological indices of increased HPA-axis reactivity, including heightened cortisol secretion in response to laboratory-induced psychosocial stress (Pruessner, Champagne, Meaney, & Dagher, 2004). Together, clinical and animal findings, such as those reported in the current study, suggest that heightened HPA-axis reactivity that manifests as a result of early-life stress contributes to the development of anorexia, in both the human population and in an animal model of ABA.

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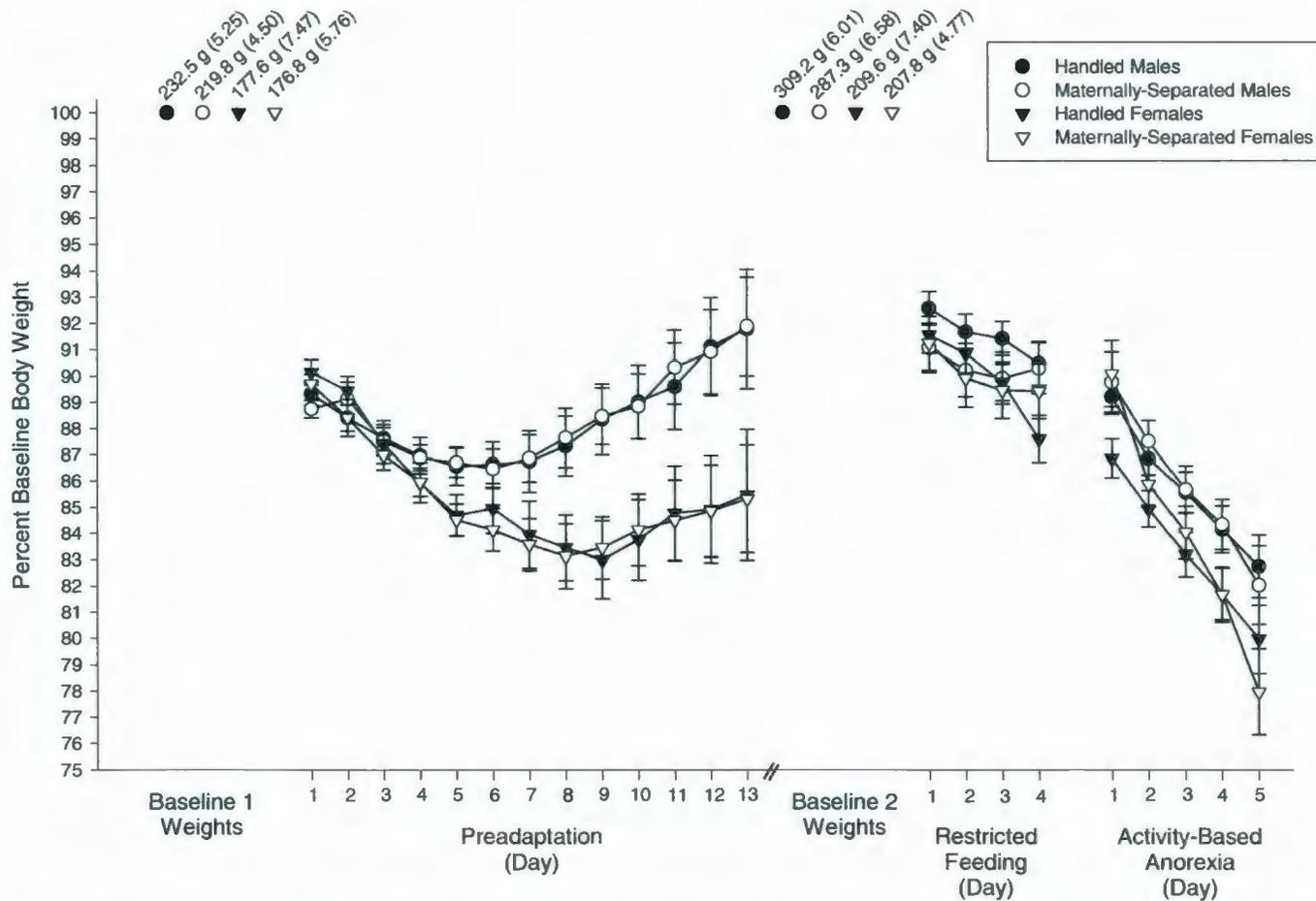


Figure 3.1. Male and female handled and maternally separated rats' body weights during two baseline recordings, percent Baseline-1 body weights during preadaptation, and percent-Baseline-2 body weights during reintroduction of restricted feeding and the ABA paradigm. Data points represent group means \pm standard error.

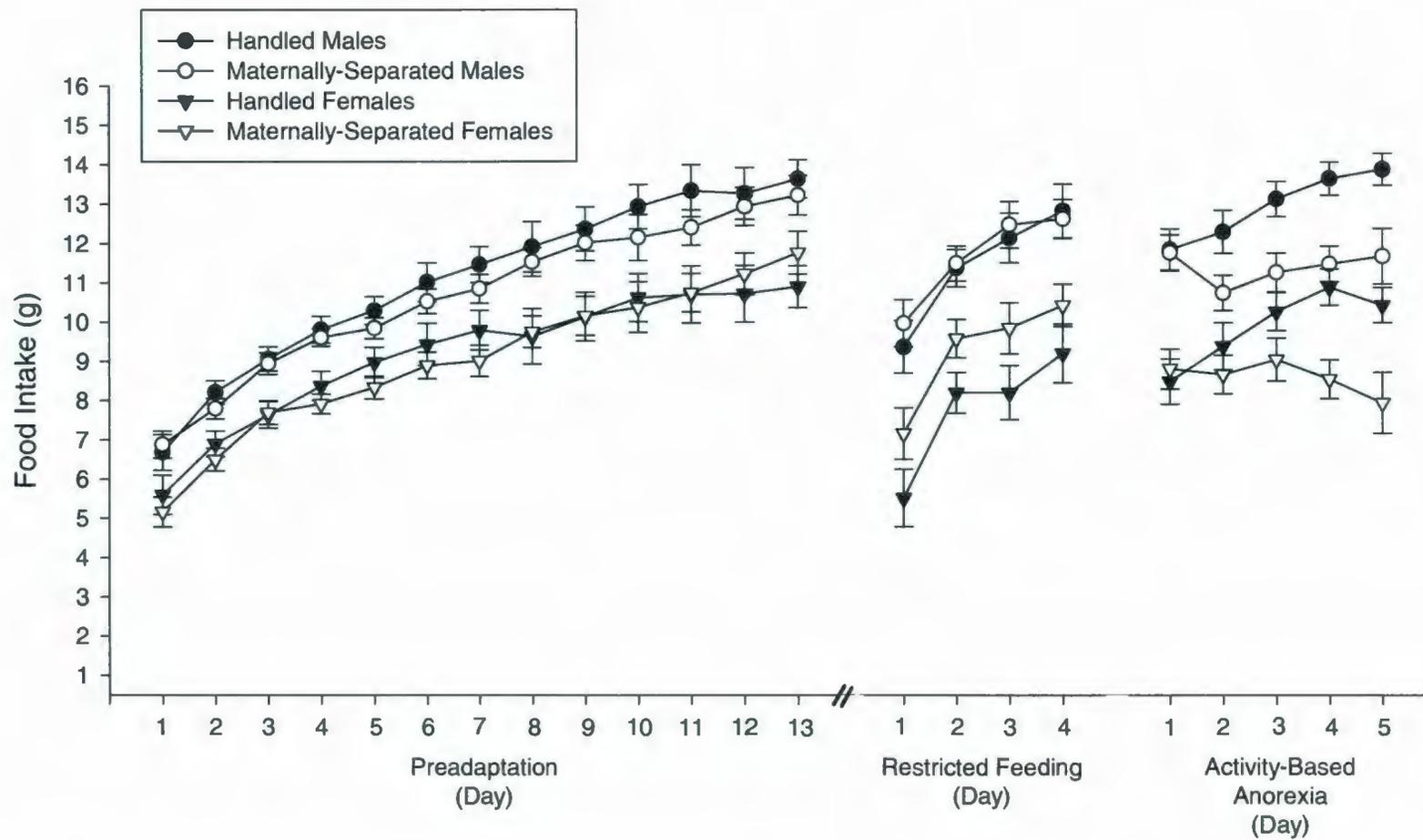


Figure 3.2. Daily food intake of male and female handled and maternally separated rats throughout preadaptation to the 1-hr restricted feeding schedule, reintroduction of restricted feeding, and the ABA paradigm. Data points represent group means \pm standard error.

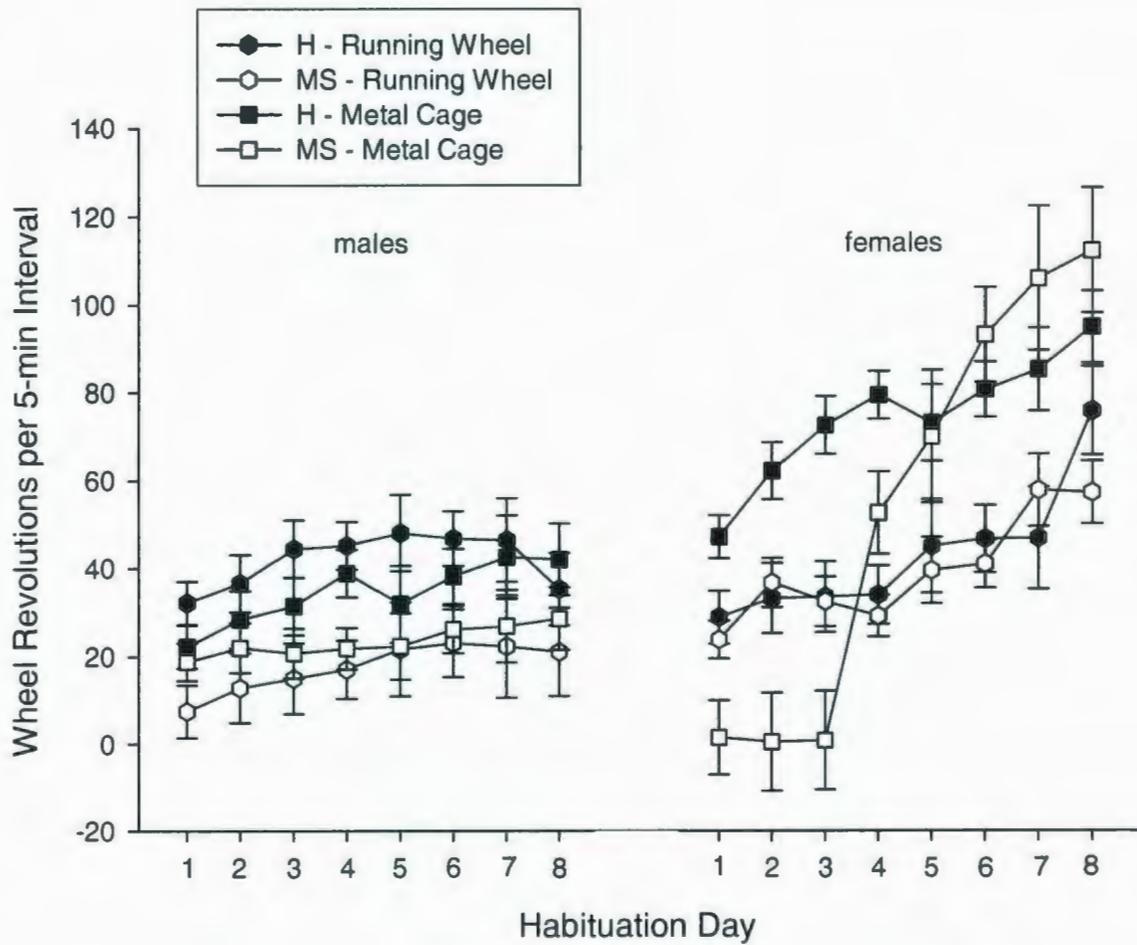


Figure 3.3. Habituation running rates in male (left-hand side) and female (right-hand side) handled (H) and maternally separated (MS) rats. On each of the 8 days, rats were exposed either to a running wheel first, followed by a metal cage (Running Wheel), or to a metal cage first, followed by a running wheel (Metal Cage). Data are portrayed across days as mean wheel revolutions per interval; cumulative daily wheel revolutions are equivalent to per-interval revolutions multiplied by 6. Data points represent group means \pm standard error.

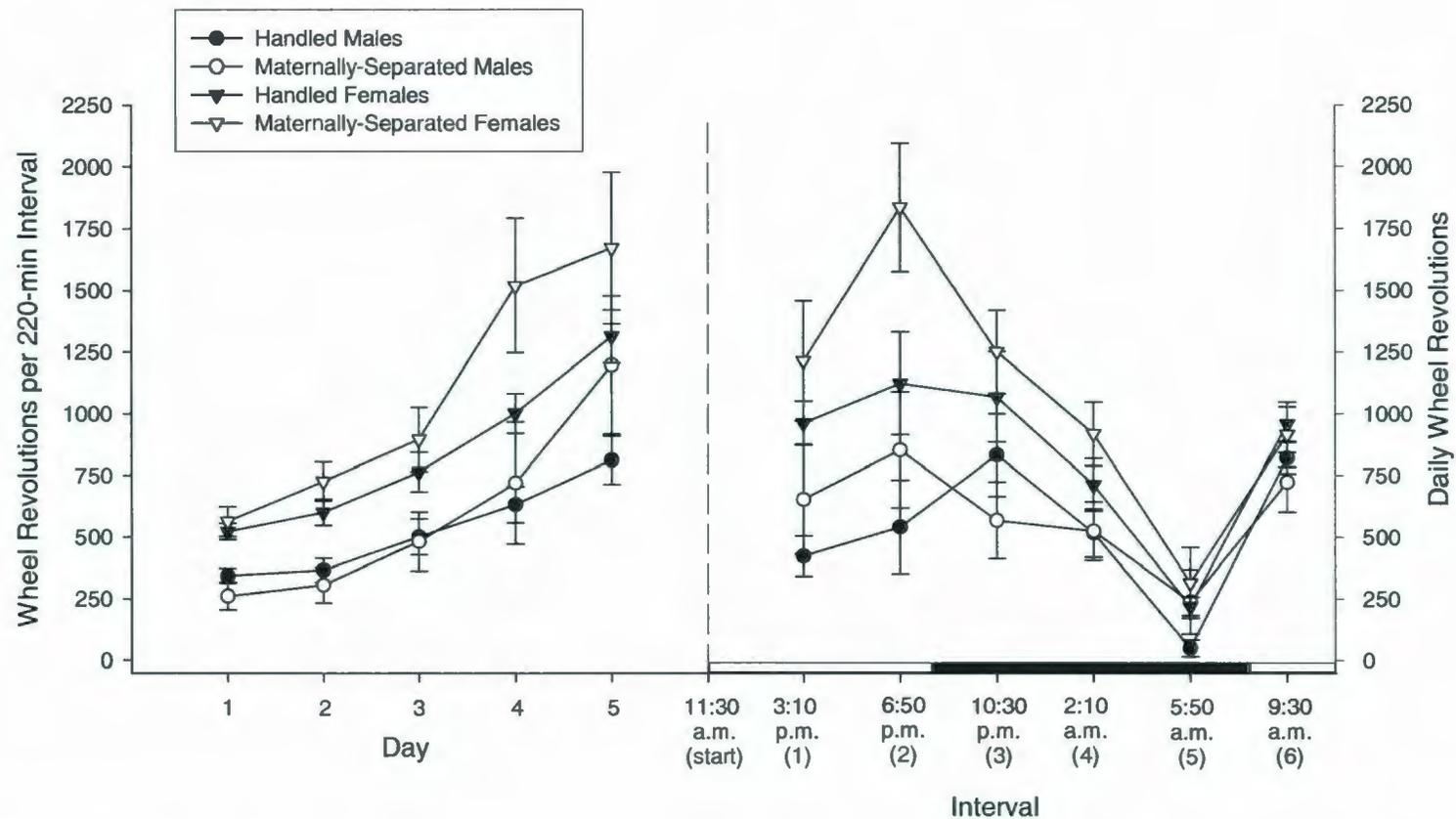


Figure 3.4. Male and female handled and maternally separated rats' running rates during the first 5 of 10 ABA days. The left-hand side portrays mean wheel revolutions per interval as a function of days. Cumulative daily wheel revolutions are equivalent to per-interval revolutions multiplied by 6. The right-hand side illustrates mean daily wheel revolutions during each 220-min interval beginning at 11:30 a.m. and continuing until 9:30 a.m. on the following day. Data points represent group means \pm standard error.

Chapter 4: Sexually Dimorphic Effects of Postnatal Treatment on the Development of Activity-Based Anorexia in Adolescent and Adult Rats

4.1 Abstract

Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis is a marked feature of anorexia nervosa. Using the activity-based animal model of anorexia nervosa (ABA), we examine whether factors known to affect HPA axis activity influence the development of ABA. Male and female rats were subjected to maternal separation or handling procedures during the first two postnatal weeks and tested in a mild version of the ABA paradigm either in adolescence or adulthood. Compared to handled females, maternally separated females demonstrated greater increases in wheel running and a more pronounced running-induced suppression of food intake during adolescence, but not in adulthood. In contrast, it was only in adulthood that wheel running produced more prolonged anorexic effects in maternally separated than in handled males. These findings highlight the interplay between early postnatal treatment, sex of the animal, and developmental age on running, food intake, and rate of body weight loss in the ABA paradigm.

4.2 Introduction

Anorexia nervosa is an eating disorder characterized by dietary self-restriction (World Health Organization, 2007), below-normal body weight for one's age and height (American Psychiatric Association, 1994), elevated levels of physical activity (Casper, 2006), and disturbances in endocrine, metabolic, and other bodily functions (Kaye, 2008; World Health Organization, 2007). Anorexia is familial, suggesting a genetic tendency that, through interplay with environmental factors, predisposes an individual to develop the disorder (Bulik, 2005). The precise nature of the gene-environment interaction is, however, difficult to untangle (Klump & Gobrogge, 2005). Heightened anxiety and incidence of anxiety-related disorders often precede the development of anorexia nervosa (Swinbourne & Touyz, 2007). Further, early-life experiences, including insecure attachment (Connan, Campbell, Katzman, Lightman, & Treasure, 2003; Ward, Ramsay, & Treasure, 2000) and stressful events in childhood and adolescence (Donohoe, 1984; Lo Sauro, Ravaldi, Cabras, Faravelli, & Ricca, 2008), are recognized as contributing factors. These findings accord with the higher prevalence of anorexia nervosa in teenage girls (Hoek & van Hoeken, 2003); not only does anxiety peak in adolescence (Buchanan, Eccles, & Becker, 1992), but teenage girls, in particular, report more stressful experiences than do teenage boys or older women and men (Ge, Lorenz, Conger, Elder, & Simons, 1994; Wagner & Compas, 1990). Thus, an individual's gender, age, life experiences, and other psychosocial and biological factors affect one's susceptibility to anorexia.

Though it is improbable that an animal model can fully encompass the complexity of variables that contribute to anorexia nervosa, the activity-based anorexia (ABA) model (Epling & Pierce, 1984, 1988; Epling, Pierce, & Stefan, 1983) is highly valid in that it reproduces many of the core behavioural and physiological features of the disorder (Casper, Sullivan, & Tecott, 2008; Hancock & Olmstead, in press). In this model, a reciprocal relationship exists between food intake and physical activity whereby food restriction leads to increases in wheel running (Finger, 1951; Moskowitz, 1959) and wheel running decreases food intake (Epling & Pierce, 1988). Under conditions of limited food availability and unlimited running-wheel access, eating is suppressed, wheel running increases, body weight drops, and animals may die (Routtenberg, 1968).

In humans (Johnson, Mastropaolo, & Wharton, 1972) and other animals (Epling & Pierce, 1988), exercise-induced suppression of food intake may result from increased activity of the hypothalamic-pituitary-adrenal (HPA) stress axis and resultant secretion of anorexigenic (appetite-suppressant) peptides, including corticotropin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and glucocorticoids (namely cortisol in humans and corticosterone in rats) (Elias et al., 1991; Farrell, Garthwaite, & Gustafson, 1983; Fediuc, Campbell, & Riddell, 2006; Wong, Licinio, Gold, & Glowa, 1993). HPA axis hyperactivity is a marked feature of anorexia; cerebrospinal fluid CRH levels are raised, as are basal levels of plasma cortisol (Gwirtsman et al., 1989; Hotta et al., 1986; Kaye, 2008; Kaye et al., 1987; Licinio, Wong, & Gold, 1996). Some studies suggest that HPA axis activity normalizes, to a certain degree, with recovery from anorexia (Doerr, Fichter, Pirke, & Lund, 1980; Kaye et al., 1987; Licino et al., 1996) whereas others

demonstrate continued HPA axis dysfunction following weight gain or full recovery (Connan et al., 2007; Ward, Brown, Lightman, Campbell, & Treasure, 1998). It is unclear, therefore, whether the HPA axis hyperactivity associated with anorexia: (a) precedes the disorder and exacerbates its symptomology; (b) is purely a consequence of the increased physical activity and self-starvation that characterize anorexia; (c) persists following full recovery as a result of permanent changes effected during the anorexic state; or (d) results from some combination of these possibilities.

Laboratory studies show that experimental manipulations that increase HPA axis reactivity, including early maternal separation (Hancock & Grant, in press), precocious weaning (Glavin & Paré, 1985), and social isolation during the juvenile and adolescent periods (Ness, Marshall, & Aravich, 1995), render animals more susceptible to ABA in later life. This suggests that heightened stress reactivity may be a risk factor for the development of anorexia, rather than a mere consequence of the disorder. Naturally-occurring variations in HPA axis reactivity, brought about by factors such as developmental age and sex of the animal, may produce similar results. In agreement with clinical findings, peak vulnerability to the behavioural and physiological effects of stress occurs in rats during the adolescent period when neural regions and neurotransmitter and hormone systems that modulate HPA axis activity undergo developmental change (Bailey & Kitchen, 1987; Rivier, 1989; Spear, 2000). Adolescence also marks the emergence of increased female susceptibility to stress (Gabriel, Roncancio, & Ruiz, 1992; Ramaley, 1972; Sencar-Cupovic & Milkovic, 1976), likely influenced by the suppressive and facilitatory effects of male and female sex hormones, respectively, on HPA axis activity

(Handa, Burgess, Kerr, & O'Keefe, 1994; McCormick, Linkroum, Sallinen, & Miller, 2002; McCormick & Mathews, 2007). Postpubertal females' basal and stress-induced levels of CRH, ACTH, and corticosterone are significantly higher than those of males (Critchlow, Liebelt, Bar-Sela, Mountcastle, & Lipscomb, 1963; Galea et al., 1997; Iwasaki-Sekino, Mano-Otagiri, Ohata, Yamauchi, & Shibasaki, 2009; Kitay, 1961; Lesniewska, Nowak, & Malendowicz, 1990). These physiological findings accord with the greater running activity of female rats, compared to males (Eikelboom & Mills, 1988; Richter, 1927; Wang, 1923), and of adolescents, compared to adults (Jones, Kimeldorf, Rubadeau, & Castanera, 1952; Woods & Routtenberg, 1971), given that stress hormone levels correlate positively with wheel running intensity (Girard & Garland, 2002; Leshner, 1971; Malisch et al., 2007; Richter, 1936).

Most ABA research has been done using male rats as subjects, despite the clinical population being comprised mainly of teenage girls and young women. Given their increased propensity for wheel running and heightened HPA axis activity, female rats might be expected to demonstrate greater vulnerability to ABA procedures than males. Yet, animal studies examining sex differences in ABA susceptibility have yielded conflicting evidence that includes: (a) no differences between young adult males and females in the percentage or rate of body weight loss or in food intake expressed as a percentage of body weight (Boakes, Mills, & Single, 1999); (b) no differences between young adult males and females in food intake, body weight loss, degree of stomach ulceration, or survival time using initial body weight as a covariate (Lambert & Kinsley, 1993); (c) decreased food intake and time required to reach a weight-loss-based removal

criterion in adolescent male rats (Doerries, Stanley, & Aravich, 1991); (d) shorter survival time for female rats of an unspecified age (Manning, Wall, Montgomery, Simmons, & Sessions, 1978; Paré, Vincent, Isom, & Reeves, 1978); and (e) increased rate of body weight loss and decreased food intake and time required to reach a weight-loss-based removal criterion in adult female rats (Hancock & Grant, in press). Given the methodological differences and varying ages of animals used in these studies, it is difficult to draw firm conclusions regarding the effect of sex on susceptibility to ABA or how developmental age may influence any sex-based differences.

In the current study, we examine whether early-life maternal separation, known to increase HPA axis reactivity (Plotsky & Meaney, 1993), differentially influences the development of ABA in male and female rats introduced to wheel running and food restriction in adolescence or adulthood. We expected that the low initial body weights of adolescent animals might expedite running-induced weight loss and removal from the experiment (Carrera, Gutierrez, & Boakes, 2006; Paré, 1975), preventing an analysis of between-group differences across days. Therefore, we adapted the typical ABA procedure so that animals allocated to running wheels were allowed only 2 h of wheel access followed by 1 h of food availability. This shortened duration of wheel running has been shown to produce suppression of food intake and reduction of body weight characteristic of ABA (Boakes & Dwyer, 1997; Epling & Pierce, 1984), but to a lesser extent than that produced by unlimited wheel access (Epling & Pierce, 1984). Animals allocated to the non-running control condition were placed for 2 h in small metal cages with similar tactile and space-confining properties as those of the running wheels.

Although anorexia may be at least partially dependent upon genetic predisposition, there is no genetic model of anorexia in rats that replicates symptoms of the clinical population (Hancock & Olmstead, in press). We therefore attempted to control for the effects of genetics in the current study by breeding pairs of sisters with one unrelated male and allocating offspring of each sister to different postnatal treatment groups, either handled or maternally separated (see Method for details). Based primarily on the physiological evidence reviewed above, we hypothesized that increased susceptibility to ABA would manifest in maternally separated compared to handled animals, in adolescents compared to adults, and in females compared to males. We also expected that these factors might interact to produce a particularly strong anorexic effect in adolescent maternally separated females.

4.3 Method

4.3.1 Subjects

4.3.1.1 Breeding

During a seven-day breeding period, Sprague-Dawley rats were housed so that virgin sisters shared a single cage with one unrelated male. Three days after the breeding period, eight pregnant rats (four pairs of sisters) were transported from Memorial University's animal facility at approximately 5 to 8 days gestation (estimated post hoc based on date of birth). Two of the four pairs of sisters were mated and transported from the animal facility after offspring from the first two pairs had completed experimental procedures, due to limited availability of equipment. On arrival, each of the eight females was singly housed in an opaque polycarbonate birth cage (48 cm × 38 cm × 20 cm) lined

with 4 cm of woodchip bedding (Sani-chips[®]; P.J. Murphy Forest Products, Montville, N.J., U.S.A.) and kept in a temperature-controlled ($22 \pm 1^\circ\text{C}$) holding room with a 12-h light/dark cycle (lights on at 8:00 a.m.). Food (Prolab[®] Rat/Mouse/Hamster 3000 5P00; LabDiet[®]) and water were available ad libitum. Cage bedding was unchanged throughout the remainder of pregnancy to avoid stressing the dams. Beginning 19 days following the start of the breeding period, dams were checked for parturition at 9:00 a.m. and 5:00 p.m. daily with day of birth designated postnatal day (PND) 0.

4.3.1.2 Postnatal Treatment

Four litters [sizes (female:male ratio): 14 (1.3), 17 (0.7), 17 (0.6), and 18 (2.0) offspring/litter], consisting of pups from one sister of each pair, were designated handled. The remaining four litters [sizes (female:male ratio): 16 (1.0), 16 (0.8), 17 (1.8), and 19 (0.7) offspring/litter], consisting of pups from the alternate sister of each pair, were designated maternally separated. On each of PND 1 through PND 14, inclusive, each dam was removed from her birth cage and placed individually in a designated polycarbonate holding cage (45 cm \times 24 cm \times 20 cm; lined with 2 cm of woodchip bedding) with free access to food and water. Pups were subsequently removed from their birth cage by a gloved experimenter and each litter was placed individually in a plastic, paper-towel lined container (26.5 cm \times 18.5 cm \times 11.0 cm with 1.0 cm \times 1.5 cm square openings spaced 0.75 cm apart along the four walls). Each container of pups was transported to an adjacent room and placed in an incubator (Nursery Hospital 1 Mechanical Brooder; internal measurements of 30 cm \times 30 cm \times 30 cm) maintained at 55-58% humidity and nest-like temperatures of 33-34 $^\circ$ Celsius on PND 1 to 6, and 31-32 $^\circ$ Celsius on PND 7 to

14 (Jans & Leon, 1983; Jans & Woodside, 1990). Pups remained apart from their dam for either 15 min, from 9:45 a.m. to 10:00 a.m. (handled group), or for 180 min, from 10:00 a.m. to 1:00 p.m. (maternally separated group). Each litter occupied one of eight containers which, along with its paper-towel lining, remained unchanged and unshared by any other litter during postnatal treatment procedures.

To avoid effects of litter size and sex ratios on adolescent and adult weights, food consumption, and anxiety-like behaviours (Dimitsantos, Escorihuela, Fuentes, Armario, & Nadal, 2007; Seitz, 1954), litters were standardized to 12 pups (6 males and 6 females) on PND 1 during the period away from the dam. Also during this time, one-third of the woodchip bedding was removed from each birth cage and replenished with fresh bedding. Following the 15-min (handling) or 180-min (maternal separation) period, pups were immediately returned to their designated birth cage, rolled in bedding, and placed back into the area of the cage from which they had been retrieved by the experimenter; the dam was then returned to her pups. On PND 14 during handling or maternal separation, two-thirds of the woodchip bedding was removed from each birth cage and replenished with fresh bedding. Litters were then left undisturbed until weaning on PND 21 when animals were housed in groups of 3 with same-sex littermates in clear polycarbonate holding cages (45 cm × 24 cm × 20 cm; lined with 3 cm of woodchip bedding) and provided ad libitum access to food and water.

4.3.2 Apparatus

There were 12 small metal cages and 12 running wheels. The small metal cages (18 cm × 25 cm × 18 cm) had a floor and front wall constructed of wire mesh (0.2 cm

wire spaced 1.1 cm apart) and the remaining three walls and lid of sheet metal. Running wheels measured 11.5 cm in width with a circumference of 113.1 cm. The floor of each running wheel was constructed of wire mesh (0.2 cm wire spaced 1.1 cm apart) and the side walls of sheet metal. Attached to the edge of the rotating wall of the wheel was a small magnet that rotated during wheel revolutions. Extending from the opposite, stationary wall were two arms spaced 180° apart, each equipped with a magnetic counter. A wheel revolution was recorded by a computer once the magnet had made a full 360° turn past both counters. Entry to and exit from the wheel was via an opening (11.7 cm × 7.5 cm) in the stationary wall across which a sliding metal door was placed during wheel running. Feeding tests were conducted in clear polycarbonate feeding cages (45 cm × 24 cm × 20 cm), similar to the home cage except that the floor was left bare to allow for the recovery and weighing of uneaten food crumbs.

4.3.3 Procedure

On PND 32, pups were separated according to sex into two holding rooms where light cycle, temperature, and humidity levels accorded with those of the original holding room. Two handled and two maternally separated litters were assigned to the adolescent group (N = 48) and the remaining two handled and two maternally separated litters to the adult group (N = 48). Two female adolescent animals were removed from the experiment prior to its completion due to health concerns and are not included in the analyses. The total number of adolescent animals was thereby reduced to 46 (24 males; 22 females).

To diminish the effects of novelty-induced anxiety and resultant variations in locomotor activity and eating behaviours, animals were habituated to the apparatuses

used in the ABA procedure. Animals continued to have free access to food and water in the homecage, but not in the apparatuses, during this period. Adolescent and adult groups experienced a total of 16, 30-min exposures to the apparatuses: 8 to a running wheel and 8 to a metal cage. All sessions were conducted between 9:30 and 10:30 a.m. The adolescent group was habituated to the apparatuses from PND 34 to 41, inclusive, with one running-wheel and one metal-cage exposure occurring in immediate succession on each day. For the adult group, habituation procedures were spread across the adolescent period with either a running-wheel or metal-cage exposure taking place on a single day. From PND 36 to 41, the adult group experienced three running-wheel and three metal-cage exposures that alternated daily. From PND 47 to 51, the adult group experienced five consecutive daily exposures to either a running-wheel or metal-cage and were switched to the alternate apparatus for exposures throughout PND 54 to 58. Scheduling habituation sessions in this way ensured that adult animals were handled by the experimenter and experienced the same number of apparatus exposures at ages that approximated those during which the adolescent group underwent habituation and ABA procedures. The order of running-wheel and metal-cage exposures was counterbalanced across male and female, handled and maternally separated animals.

On PND 43 (adolescent group) or PND 74 (adult group), Baseline body weights were recorded and all food was removed from the homecage at 12:00 noon; water access remained ad libitum. At 12:00 noon on the following day, rats were removed from the homecages, Restriction body weights were recorded, and animals were subjected to a 1-h feeding test, as follows: each rat was individually placed in a feeding cage with free water

access and approximately 30 g of the animal's regular food located in the feeder section of the wire cage lid. Rats were removed from the feeding cages at 1:00 p.m. and placed back in the homecages. Uneaten food was retrieved from the lid of the feeding cage, as were any food crumbs present on the cage bottom, and reweighed. Using body-weight and feeding-test data, animals were evenly matched and assigned to one of two apparatus groups: the metal cage group or the running wheel group. Groups were balanced so that each contained equal numbers of male and female, handled and maternally separated offspring, and so that half of each litter was assigned to each apparatus. At 10:00 a.m. on the following day (PND 45 for adolescents; PND 76 for adults), rats were removed from the homecages and weighed. Then, animals allocated to the metal cage group were placed individually within metal cages whereas those allocated to the running wheel group were placed individually within running wheels for a 2-h period. For animals assigned to the running wheel group, wheel revolutions were recorded in six, 20-min bins across the 2 h of wheel running. At 12:00 noon, animals were removed from their respective apparatus and placed individually within feeding cages for a 1-h feeding test, as previously described. At 1:00 p.m., animals were removed from the feeding cages and returned to the homecages. This procedure continued daily for a total of 12 consecutive days, or until animals met the removal criterion (defined below), at which point they were returned to ad libitum feeding and procedures were halted. Experimental procedures adhered to the regulations set out by the Canadian Council on Animal Care and were approved and monitored by the Animal Care Committee.

4.4 Results

The following results include analyses of body weights, daily food intake, and wheel revolutions across intervals and days, between male and female, handled and maternally separated animals. Because these measures vary as a function of rat age (Hubert, Laroque, Gillet, & Keenan, 2000; Mondon, Dolkas, Sims, & Reaven, 1985), data were similarly but separately analyzed for adolescent and adult animals. When significant interactions are reported, post hoc *p* values were obtained from analyses of least significant difference. Adaptation was measured according to a survival criterion initially established by Routtenberg (1968) and since employed by others (e.g., Dwyer & Boakes, 1997; Lett, Grant, Smith, & Koh, 2001); an animal is considered to have successfully adapted when its body weight on the fourth day of any consecutive 4-day period is equal to or greater than its weight on the first day of that period. Removal criterion was reached when an animal's body weight fell to 75% that of Baseline body weight. Variables discriminating between animals that adapted, were removed, or failed to reach either criterion were also assessed.

4.4.1 Body Weights

Baseline body weights (g) were analyzed using a 2 (postnatal treatment) \times 2 (sex) analysis of variance (ANOVA). A similar ANOVA was conducted on Restriction body weights, analyzed as percentage of Baseline body weights. During the ABA procedure, percentage of Baseline body weights were assessed across the period during which none of the animals had reached the removal criterion. Within the adolescent group, the first animal fell to 75% Baseline body weight at the start of ABA day 5. A 2 (postnatal treatment) \times 2 (sex) \times 2 (apparatus) \times 5 (day) repeated measures ANOVA, with day as

the repeated factor, was applied to the adolescent body weight data. Within the adult group, no animal met the removal criterion and procedures were conducted for 12 days, the duration of time required for all adolescent animals to reach either the removal or adaptation criterion. A 2 (postnatal treatment) \times 2 (sex) \times 2 (apparatus) \times 12 (day) repeated measures ANOVA, with day as the repeated factor, was applied to the adult body weight data. Adolescent group body weights are presented in Figure 4.1 and adult group body weights in Figure 4.2.

4.4.1.1 Adolescent Group

At Baseline, males weighed significantly more than females, $F(1, 42) = 243.99, p < .01$, with no effect of postnatal treatment on body weights, $F(1, 42) = 3.08, p = .09$, and no Postnatal Treatment \times Sex interaction, $F < 1$. Following 24-h food restriction (R in Figure 4.1), males' percentage of Baseline weights fell below those of females, $F(1, 42) = 6.79, p < .05$. There continued to be no weight differences between handled and maternally separated animals, $F(1, 42) = 2.34, p = .13$, and no two-way interaction, $F < 1$. During the ABA procedure, there was a steady decrease in body weight across days, $F(4, 152) = 137.47, p < .01$. Running wheel animals lost a greater percentage of weight than did metal cage animals, $F(1, 38) = 8.43, p < .01$, and showed accelerated weight loss across days, $F(4, 152) = 16.41, p < .01$, so that they were lighter than metal cage animals on ABA days 3 through 5 (p values $\leq .01$). Whereas there was no main effect of postnatal treatment, $F(1, 38) = 1.33, p = .26$, or of sex, $F(1, 38) = 2.42, p = .13$, there were both Postnatal Treatment \times Day, $F(4, 152) = 3.13, p < .05$, and Sex \times Day, $F(4, 152) = 5.27, p < .01$, interactions. From ABA day 1 to 5, maternally separated animals lost an average of

5.82% body weight whereas handled animals lost only 4.38%. Over the same period, females lost an average of 5.95% body weight whereas males lost 4.25%. Finally, although it appears in Figure 4.1 that maternally separated running wheel animals lost a greater percentage of body weight compared to both their metal cage counterparts and the handled running wheel group, the three-way Postnatal Treatment \times Apparatus \times Day interaction fell just short of significance, $F(4, 152) = 2.31, p = .06$. All remaining two-, three-, and four-way interactions were non-significant (p values $> .22$).

4.4.1.2 Adult Group

At Baseline, males' weights were greater than those of females, $F(1, 44) = 642.75, p < .01$, with no effect of postnatal treatment on body weights, $F(1, 44) = 2.27, p = .14$, and no Postnatal Treatment \times Sex interaction, $F < 1$. Following 24-h food restriction (R in Figure 4.2), males' percentage of Baseline weights were higher than those of females, $F(1, 44) = 12.30, p < .01$. There continued to be no weight differences between handled and maternally separated animals and no two-way interaction, $F_s < 1$. During the 12 days of ABA procedures, there was a steady decrease in body weights, $F(11, 440) = 166.57, p < .01$, until day 10 when weights stabilized (p values $> .29$). Overall, weights were lower in running wheel than in metal cage animals, $F(1, 40) = 8.33, p < .01$, but this effect varied across days, $F(11, 440) = 10.33, p < .01$. Metal cage animals' body weights remained stable from ABA day 7 onward (p values $> .13$) whereas running wheel animals' weights did not stabilize until ABA day 10 (p values $> .18$). Running wheel animals' percentage of Baseline body weight fell significantly lower than that of metal cage animals on ABA days 5 through 12 (p values $< .05$). There was no

main effect of sex on percentage Baseline weights, $F < 1$, but there was a significant Sex \times Day interaction, $F(11, 440) = 3.40, p < .01$. From ABA day 1 to 12, males lost an average of 8.01% weight from Baseline whereas females lost an average of 5.93%. While there was no Sex \times Apparatus interaction, $F < 1$, there was a three-way Sex \times Apparatus \times Day interaction, $F(11, 440) = 1.88, p < .05$. Within males, percentage Baseline body weights of running wheel animals dropped below those of metal cage animals from ABA day 6 onward (p values $\leq .05$). Within females, running wheel animals weighed significantly less than metal cage animals from ABA day 9 onward (p values $< .05$). In contrast to adolescent group data, there was no main effect of postnatal treatment on body weights and no two-, three-, or four-way interactions involving postnatal treatment, all F values < 1 .

4.4.2 Food Intake

Following 24 h of food restriction, intake during a 1-h feeding test was analysed separately in adolescent and adult groups using 2 (postnatal treatment) \times 2 (sex) ANOVAs. During the ABA paradigm, feeding-test intake was measured daily and analyzed across the period during which all animals remained in the experiment. For adolescent animals, a 2 (postnatal treatment) \times 2 (sex) \times 2 (apparatus) \times 4 (day) repeated measures ANOVA, with day as the repeated factor, assessed food intake. For adult animals, the analysis consisted of a 2 (postnatal treatment) \times 2 (sex) \times 2 (apparatus) \times 12 (day) repeated measures ANOVA, with day as the repeated factor. Food intake results are presented in Figure 4.3 for the adolescent group and in Figure 4.4 for the adult group.

4.4.2.1 Adolescent Group

Post-restriction intake (R in Figure 4.3) differed only as a function of sex with males consuming more food than females, $F(1, 42) = 36.12, p < .01$. There was no effect of postnatal treatment and no interaction, F values < 1 . During the first four days of the ABA paradigm, food intake increased daily, $F(3, 114) = 140.64, p < .01$ (p values $< .01$). Males continued to consume more chow compared to females, $F(1, 38) = 56.26, p < .01$, and metal cage animals ate significantly more than running wheel animals, $F(1, 38) = 8.61, p < .01$. There also emerged a significant effect of postnatal treatment whereby handled animals consumed more chow than did maternally separated animals, $F(1, 38) = 6.01, p < .05$. Although it appears from Figure 4.3 that food intake between handled and maternally separated animals differed across days, the Postnatal Treatment \times Day interaction fell just short of significance, $F(3, 114) = 2.59, p = .06$. Visual inspection of Figure 4.3 further suggests that the intake of running wheel and metal cage animals was more dissimilar in maternally separated females than in any of the other three groups. If we compare running wheel to metal cage animals' food intake using additional 2 (apparatus) \times 4 (day) repeated measures ANOVAs, the eating-suppressive effect of wheel running is apparent only within maternally separated females, $F(1, 9) = 7.60, p < .05$, and is absent in maternally separated males, $F(1, 10) = 1.69, p = .22$, handled females, $F(1, 9) = 1.11, p = .32$, and handled males, $F < 1$. All other interactions were non-significant (p values $> .31$).

4.4.2.2 Adult Group

Following 24 h of food restriction (R in Figure 4.4), males consumed more chow than did females, $F(1, 44) = 107.24, p < .01$, with no differences in intake resulting from

postnatal treatment and no two-way interaction, F values < 1 . During the 12 days of ABA procedures, food intake increased steadily across days, $F(11, 440) = 99.91, p < .01$. Males consumed more chow than did females, $F(1, 40) = 104.96, p < .01$, and metal cage animals consumed more chow than did running wheel animals, $F(1, 40) = 8.73, p < .01$. There was also an Apparatus \times Day interaction, $F(11, 440) = 2.40, p < .01$, whereby the difference in food intake between metal cage and running wheel animals became less pronounced across days. Running wheel animals were eating significantly less than metal cage animals on ABA days 1 through 8 (p values $< .05$) but eating increased to levels approximating those of metal cage animals on days 9 through 12 (p values $> .05$), suggesting that adult animals were able to overcome the eating-suppressive effects of wheel running. The Apparatus \times Day interaction also varied according to sex, $F(11, 440) = 4.23, p < .01$. In males, the anorexic effect of wheel running was apparent during the first 7 days of ABA procedures in that running wheel animals consumed significantly less chow on those days than did metal cage animals (p values $< .01$, except on ABA days 4 and 7 where $p = .06$). Compared to metal cage males' intake, which was relatively high to start and showed moderate increases across days, running wheel males' intake was lower to start and showed a steeper increase across days. This pattern resulted in no differences in intake between metal cage and running wheel males during ABA days 8 through 12 (p values $> .24$). Females, on the other hand, failed to demonstrate the typical ABA effect, showing comparable levels of food intake regardless of apparatus (except on ABA day 10, when metal cage females consumed more food than running wheel females, $p < .05$).

Both metal cage and running wheel females showed marked increases in food intake, particularly across the first 7 days of the ABA procedure.

Finally, whereas there was no main effect of postnatal treatment on food intake, $F(1, 40) = 2.35, p = .13$, there was a three-way Postnatal Treatment \times Sex \times Day interaction, $F(11, 440) = 1.95, p < .05$. On ABA day 1, food intake was much more disparate between handled males and females (mean difference = 4.68 g) than between maternally separated males and females (mean difference = 2.95 g) which appears to be a function of high intake in handled males and low intake in maternally separated males ($p = .06$) rather than differences in food intake between handled and maternally separated females ($p = .66$). This suggests that the first experience of 2-h confinement, regardless of apparatus, produced differential effects between handled and maternally separated males but not between handled and maternally separated females. Whereas maternally separated males' intake became increasingly similar to that of handled males as days progressed (p values $> .10$), maternally separated females' eating levels became increasingly dissimilar to those of handled females. Maternally separated females consumed less food than did handled females on days 5 ($p < .05$), 6 ($p < .01$), and 10 ($p < .05$) and showed a trend towards eating less on day 8 ($p = .10$). Although the four-way Postnatal Treatment \times Sex \times Apparatus \times Day interaction was non-significant, $F < 1$, visual inspection of Figure 4.4 suggests that differing levels of food intake between running wheel and metal cage conditions existed for a prolonged period in maternally separated compared to handled males. If we isolate males and run additional analyses using a 2 (postnatal treatment) \times 2 (apparatus) \times 7 (day) repeated measures ANOVA to compare maternally separated and

handled males' intake across days 1 to 7 during which the ABA effect was found, there emerges a significant Postnatal Treatment \times Apparatus \times Day interaction, $F(6, 120) = 2.47, p < .05$. Post hoc analyses show that wheel running suppressed food intake in handled males only on ABA days 1 through 3 (p values $\leq .05$) whereas maternally separated males' intake was suppressed following wheel running on all 7 days (p values $< .05$).

4.4.3 Wheel Running

Wheel revolutions were recorded daily during ABA procedures in six, 20-min bins and analyzed across the period in which no animals had reached the removal criterion. For the adolescent group, running was assessed using a 2 (postnatal treatment) \times 2 (sex) \times 4 (day) \times 6 (interval) repeated measures ANOVA, with day and interval as the repeated factors. In the adult group, the analysis consisted of a 2 (postnatal treatment) \times 2 (sex) \times 12 (day) \times 6 (interval) repeated measures ANOVA, with day and interval as the repeated factors. These data are presented in Figures 4.5 (adolescent group wheel revolutions) and 4.6 (adult group wheel revolutions).

4.4.3.1 Adolescent Group

Daily per-interval wheel revolutions increased significantly across ABA days 1 through 4, $F(3, 57) = 112.83, p < .01$ (right panel of Figure 4.5). There was no main effect of postnatal treatment, $F < 1$, or of sex, $F(1, 19) = 2.03, p = .17$, on wheel running rates, however there was a significant Postnatal Treatment \times Sex interaction, $F(1, 19) = 8.02, p < .05$. Whereas running rates did not differ between maternally separated and handled males ($p = .09$), maternally separated females ran at a higher rate than did

handled females ($p < .05$). This resulted in higher running rates in maternally separated females compared to males ($p < .01$) but similar rates in handled females compared to males ($p = .34$). There was also a three-way Postnatal Treatment \times Sex \times Interval interaction, $F(5, 95) = 3.77, p < .01$ (left panel of Figure 4.5). Handled males ran at a rate similar to that of maternally separated males during intervals 1 through 4 (p values $> .06$) but ran more than maternally separated males in intervals 5 and 6 (p values $< .05$). In contrast, handled females ran at a rate similar to that of maternally separated females only during intervals 1 and 2 (p values $> .07$); running rates were significantly lower in handled females than in maternally separated females during intervals 3 through 6 (p values $< .05$). This resulted in similar running rates between handled males and females during all intervals (p values $> .36$; except interval 6 where $p < .05$), and significantly higher running rates in maternally separated females compared to males in all intervals (p values $< .05$; except interval 1 where $p = .09$). There were no other main effects or interactions (p values $> .10$).

4.4.3.2 Adult Group

Across the 12 days of ABA procedures, there was a steady increase in per-interval wheel revolutions, $F(11, 220) = 100.86, p < .01$ (right panel, Figure 4.6), with females running at a higher rate than males, $F(1, 20) = 24.78, p < .01$. Sex differences in running rates also varied across days, $F(11, 220) = 3.59, p < .01$, with females demonstrating steeper increases in per-interval running rates across days than males. Although the effect of postnatal treatment was marginally non-significant, $F(1, 20) = 4.06, p = .06$, it did interact significantly with day, $F(11, 220) = 2.52, p < .01$. Per-interval running rates

increased more quickly in handled than maternally separated animals, but this effect was mainly due to the increase in running by the handled males, as indicated by the significant Postnatal Treatment \times Sex \times Day interaction, $F(11, 220) = 2.66, p < .01$. In males, running rates diverged across days so that handled males were running significantly more than maternally separated males on ABA days 7 through 12 (p values $< .05$, except on day 9 when $p = .11$). This pattern was absent in female animals with handled and maternally separated females' running rates similar on all days (p values $> .15$).

Running rates also changed across intervals, $F(5, 100) = 17.90, p < .01$ (left panel, Figure 4.6). Overall, animals ran at similar rates during intervals 1 through 3 (p values $> .40$) and during intervals 4 through 6 (p values $> .17$); running rates were higher in intervals 1 through 3 compared to in intervals 4 through 6 (p values $< .01$). The effect of interval varied, however, according to both sex, $F(5, 100) = 5.26, p < .01$, and postnatal treatment, $F(5, 100) = 4.93, p < .01$. Females ran at a significantly higher rate than did males across all 6 intervals (p values $< .01$), however, sex differences in running rates became less pronounced as intervals progressed. In interval 1, females' running rates were higher than those of males by an average of 181.60 revolutions; by interval 6, this difference had fallen to 109.07 revolutions. Handled and maternally separated animals ran at similar rates during intervals 1 and 2 (p values $\geq .12$). During intervals 3 through 6, handled animals ran at significantly higher rates than did maternally separated animals (p values $< .05$). Although there was no significant three-way Postnatal Treatment \times Sex \times Interval interaction, $F < 1$, visual inspection of Figure 4.6 (left panel) suggests that the

effect of postnatal treatment during intervals 3 through 6 resulted from differences in running rates of handled and maternally separated males, rather than females. If we isolate males and run additional analyses using a 2 (postnatal treatment) \times 12 (day) \times 6 (interval) repeated measures ANOVA to compare running rates of handled and maternally separated males across intervals, there emerges a significant Postnatal Treatment \times Interval interaction, $F(5, 50) = 3.95, p < .01$. Post hoc analyses indicate no effect of postnatal treatment on running rates in intervals 1 and 2 (p values $\geq .09$) but a higher rate of running in handled males across intervals 3 through 6 (p values $< .05$). When the same analyses are run isolating only females, no such effect emerges, $F(5, 50) = 1.57, p = .19$.

4.4.4 Adaptation or Removal

Of all 94 animals, 55 met the adaptation criterion, 10 met the removal criterion, and the remaining 29 failed to meet either criterion during the course of ABA procedures. Discriminant function analysis was carried out to determine which combination of the four independent variables (postnatal treatment, sex, apparatus, and age) significantly predicted membership in each of the three outcome categories. Category membership frequencies are presented in Table 4.1. The overall analysis yielded two significant functions that resulted in correct classification of 81.9% of original grouped cases and 76.6% of cross-validated grouped cases. The first significant function (Wilks' lambda = 0.35, $p < .01$) accounted for 70.5% of the variance between outcome categories; age ($r = .94$) and sex ($r = .13$) predictor variables correlated significantly with this function. Within adolescent animals, approximately 22% reached the removal criterion whereas all

remaining animals adapted. Within adult animals, none reached the removal criterion, approximately 50% of females and 25% of males reached the adaptation criterion, and all remaining animals failed to reach either criterion. The second significant function (Wilks' lambda = 0.70, $p < .01$) accounted for 29.5% of category variance; apparatus ($r = .69$) and postnatal treatment ($r = .58$) predictor variables correlated significantly with this function. The majority of handled animals and animals in the metal cage group reached the adaptation criterion during ABA procedures and none required removal from the experiment. In contrast, approximately 42% of maternally separated running wheel animals reached removal criterion with a much smaller percentage reaching the adaptation criterion. Independently, postnatal treatment (Wilks' lambda = 0.87, $p < .01$), apparatus (Wilks' lambda = 0.83, $p < .01$), and age (Wilks' lambda = 0.53, $p < .01$) were significant predictors of outcome category whereas sex was not (Wilks' lambda = 0.98, $p = .36$).

4.5 Discussion

Even under the relatively mild conditions of 2-h running wheel access in combination with a 1-h restricted feeding schedule, our animals demonstrated characteristic ABA effects: wheel running rates increased progressively across days while, at the same time, food intake and body weight were suppressed to a greater extent in running wheel than in metal cage animals. Running wheel allocation significantly predicted fewer animals reaching adaptation criterion and more animals reaching removal criterion, compared to metal cage allocation. Early-life experience, sex, and developmental age impacted susceptibility to these effects, consistent with clinical

evidence citing the relevance of these factors to the development of anorexia nervosa (Connan et al., 2003; Hoek & van Hoeken, 2003; Lo Sauro et al., 2008).

Maternal separation during the first two postnatal weeks has been shown to exacerbate the weight- and eating-suppressant effects of 22-h wheel activity in food-restricted adult animals (Hancock & Grant, in press). In the current study, similar effects emerged in adolescent animals provided only 2 h of running wheel access. During the first 5 days of ABA procedures, maternally separated adolescents showed accelerated weight loss across days with a trend towards the greatest weight loss in those allocated to running wheels. Discriminant function analysis further highlighted the impact of early-life history in that postnatal treatment in and of itself, and when combined with apparatus allocation, significantly predicted the outcome of the animals subjected to ABA procedures; all ten animals removed from experimental procedures were maternally separated running-wheel-allocated adolescents. Early-life maternal separation was also associated with an overall greater suppression of food intake that deviated increasingly across days from that of handled animals. Within-group comparisons indicated that wheel running produced a significant anorexic effect in maternally separated females whereas food intake did not differ between running wheel and metal cage conditions for handled males, maternally separated males, or handled females. Daily wheel running activity was greatest in adolescent maternally separated females whose rates were higher than those of maternally separated males and handled females. Together, these data support our hypothesis that maternal separation increases adolescent animals' susceptibility to ABA

procedures and that, at least in terms of food intake and running rates, maternally separated females are particularly vulnerable.

Given the long-lasting influence of the postnatal environment on stress reactivity (Anisman, Zaharia, Meaney, & Merali, 1998) and the increased susceptibility to ABA of adult rats subjected to a 22-h ABA paradigm after postnatal maternal separation (Hancock & Grant, in press), we hypothesized that a similar, although likely less severe, pattern of effects would emerge in adult animals subjected to a 2-h ABA paradigm. This hypothesis was only partly supported by the data. Unlike the adolescent group, rate of body weight loss during ABA procedures in adult male and female rats was unaffected by postnatal treatment. Within adult females, there were no differences in food intake during the initial days of ABA procedures. With repeated daily apparatus confinement and increasingly prolonged food restriction, maternally separated females' intake dropped below that of handled females, perhaps reflecting a chronic-stress-like effect to which females (Luine, Beck, Bowman, Frankfurt, & MacLusky, 2007) and maternally separated animals (Aisa, Tordera, Lasheras, Del Río, & Ramírez, 2008), in particular, are most vulnerable. There was, however, no effect of wheel running on food intake. There were no differences in running rates between maternally separated and handled adult females across the 12 days of wheel access. These findings were unexpected, given the higher propensity for wheel running in animals with HPA axis hyperactivity (Girard & Garland, 2002; Malisch et al., 2007) and heightened anxiety (Gelegen et al., 2007), but are consistent with evidence that the influence of the early-life environment on female animals' response to mild stress is less pronounced once females reach adulthood

(Hancock, Menard, & Olmstead, 2005; Kalinichev, Easterling, Plotsky, & Holtzman, 2002; Wigger & Neumann, 1999).

Within adult males, food intake did not differ significantly across days between maternally separated and handled animals, although handled males' intake was slightly higher following 2-h confinement on ABA day 1, regardless of apparatus allocation. The clearest indication of a postnatal treatment effect on ABA susceptibility in adult animals was demonstrated by within-group comparisons showing that running-induced suppression of eating occurred in handled males only during the first 3 days of ABA procedures, but in maternally-separated males during the first 7 days. These data suggest a maladaptive influence of maternal separation in that, despite relatively low levels of running activity, maternally separated rats took significantly longer to increase food consumption to a level approximating that of metal cage animals whereas handled males, despite high rates of running, were quickly able to increase food intake. In addition, these findings are consistent with existing literature that maternally separated males exhibit pronounced behavioural and physiological disruption following mild stress in adulthood (Genest, Gulemetova, Laforest, Drolet, & Kinkead, 2003; Kalinichev et al., 2002; Wigger & Neumann, 1999). Unexpectedly, maternally separated males exhibited a slower increase in running across days, resulting in lower rates compared to those of handled males during the second half of ABA procedures. This was unanticipated given the higher HPA axis reactivity of adult maternally separated versus handled males following mild stress (Ladd, Thirivikraman, Huot, & Plotsky, 2005; Lippmann, Bress, Nemeroff, Plotsky, & Monteggia, 2007). That maternally separated males also showed the lowest

running rates of all groups during adolescence suggests that this pattern is dependent more on postnatal treatment than on developmental age, although these factors may be synergistic. Analysis of variables that influence voluntary running (Sherwin, 1998) suggests the importance of testosterone as wheel running rates decrease relative to the proportion of testicular tissue removed and are almost completely eradicated with a total loss of testosterone (Gans, 1927; Richter, 1927). The most pronounced rise in testosterone levels occurs in male rats between 50 and 60 days of age (Ojeda & Urbanski, 1994), although an acute testosterone peak also occurs much earlier, on the second day of life (Anderson, Fatinikun, & Swift, 1982). Removal of male pups from their dam during the early postnatal period is associated with a disruption of the testosterone peak, possibly leading to altered brain organization (Anderson et al., 1982). This possibility is supported by evidence that maternally separated adult males show lower stress-induced testosterone levels coincident with heightened corticosterone and ACTH release (Veenema, Blume, Niederle, Buwalda, & Neumann, 2006). Although speculative, this decrease in reactivity of testosterone to stress may play a role in the lower wheel running rates of maternally separated males, especially during adulthood when sex hormone systems are fully functional (Ojeda & Urbanski, 1994).

Comparing patterns of results across age groups demonstrates a more pronounced impact of ABA procedures in adolescent compared to adult animals which was likely mediated by heightened HPA axis reactivity. Compared to adults, adolescent animals demonstrate more immobility during a forced swimming test (Walker, Trottier, Rochford, & Lavallée, 1995), increased hyperphagia following tail pinch (cited in Heinrichs et al.,

1992), greater suppression of food intake, body weight gain, and time spent on the open arms of an elevated plus maze following repeated social stress (Stone & Quartermain, 1998), and increased ACTH and corticosterone release following physical restraint (Gomez, Houshyar, & Dallman, 2002). In the ABA paradigm, an important additional factor that must be considered when comparing across developmental ages is that of initial body weight. When variables such as age and housing conditions are held constant, lighter animals lose weight more quickly under ABA conditions than do heavier animals (Boakes & Dwyer, 1997). In the current study, the lightest animals (adolescent females) demonstrated the greatest reductions in body weight during ABA procedures. In addition, a greater amount of raw weight must be lost or, following initial loss, regained for heavier animals to reach the removal or adaptation criterion, respectively. This could explain why all adolescent animals were able to reach either adaptation or removal criterion whereas most adult animals, especially adult males, failed to reach either criterion. However, initial body weight does not always predict ABA susceptibility. In a 22-h wheel access ABA procedure, adolescent males were more vulnerable to running-induced body weight loss than weight-matched adult females (Boakes, Mills, & Single, 1999). Although males of most mammalian species are heavier than same-aged females (Aschkenasy-Lelu & Aschkenasy, 1959), sex did not independently predict ABA outcome in the current study. Adult males, despite weighing almost twice as much as adult females, lost a significantly greater percentage of weight during ABA procedures. Further, within both the adolescent and adult groups, there were no differences between maternally separated and handled males or females in Baseline body weights or

percentage body weight loss following 24-h food restriction. Yet, compared to their handled counterparts, adolescent maternally separated animals lost weight more quickly during ABA procedures whereas adult maternally separated animals did not. Therefore, although initial body weight may in some instances account for increased susceptibility to running-induced weight loss, it is not an all-encompassing explanation.

To summarize, the current study highlights the interplay between early-life environment, sex of the animal, and developmental age on running, food intake, and rate of body weight loss in a mildly-stressful version of the ABA paradigm. Adolescent maternally separated females demonstrated greater increases in wheel running, relative to their handled counterparts, and a more pronounced running-induced suppression of food intake; adult maternally separated and handled females did not differ on these variables. Adolescent maternally separated males failed to demonstrate running-induced suppression of food intake compared to their metal cage counterparts, but this effect was clearly present in adult maternally separated males. These data suggest that, under the mildly-stressful conditions of the current study, maternal separation was most detrimental to females during adolescence and to males during adulthood. This is an important finding considering that the effects of maternal separation are most often examined in adult males. The physiological basis for these differences is unknown and further studies are needed to confirm and extend these findings. Sex hormones are likely candidates for investigation as their activity is affected by age (Ojeda & Urbanski, 1994) and early postnatal events (Cameron et al., 2008; Veenema et al., 2006) and exerts opposing effects on HPA axis activity in male and female animals (Handa, Burgess, Kerr, & O'Keefe,

1994). In addition, because anorexia nervosa is most prevalent in adolescent girls with a history of early-life stressful events, this study provides support for the validity of the ABA paradigm as an appropriate animal model to investigate anorexia nervosa.

4.6 References

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Table 4.1: *Number of Animals that Reached Adaptation Criterion, Removal Criterion, or Neither During ABA Procedures According to Significant Predictor Variables of Category Membership.*

Predictor Variable	Group	Outcome		
		Adapted	Removed	Neither
Age × Sex	Adolescent Male	19	5	0
	Adolescent Female	17	5	0
	Adult Male	6	0	18
	Adult Female	13	0	11
Postnatal Treatment × Apparatus	Handled Running Wheel	15	0	9
	Handled Metal Cage	18	0	5
	Mat. Sep. Running Wheel	5	10	9
	Mat. Sep. Metal Cage	17	0	6
Age	Adolescent	36	10	0
	Adult	19	0	29
Postnatal Treatment	Handled	33	0	14
	Mat. Sep.	22	10	15
Apparatus	Running Wheel	20	10	18
	Metal Cage	35	0	11
Sex	Male	25	5	18
	Female	30	5	11

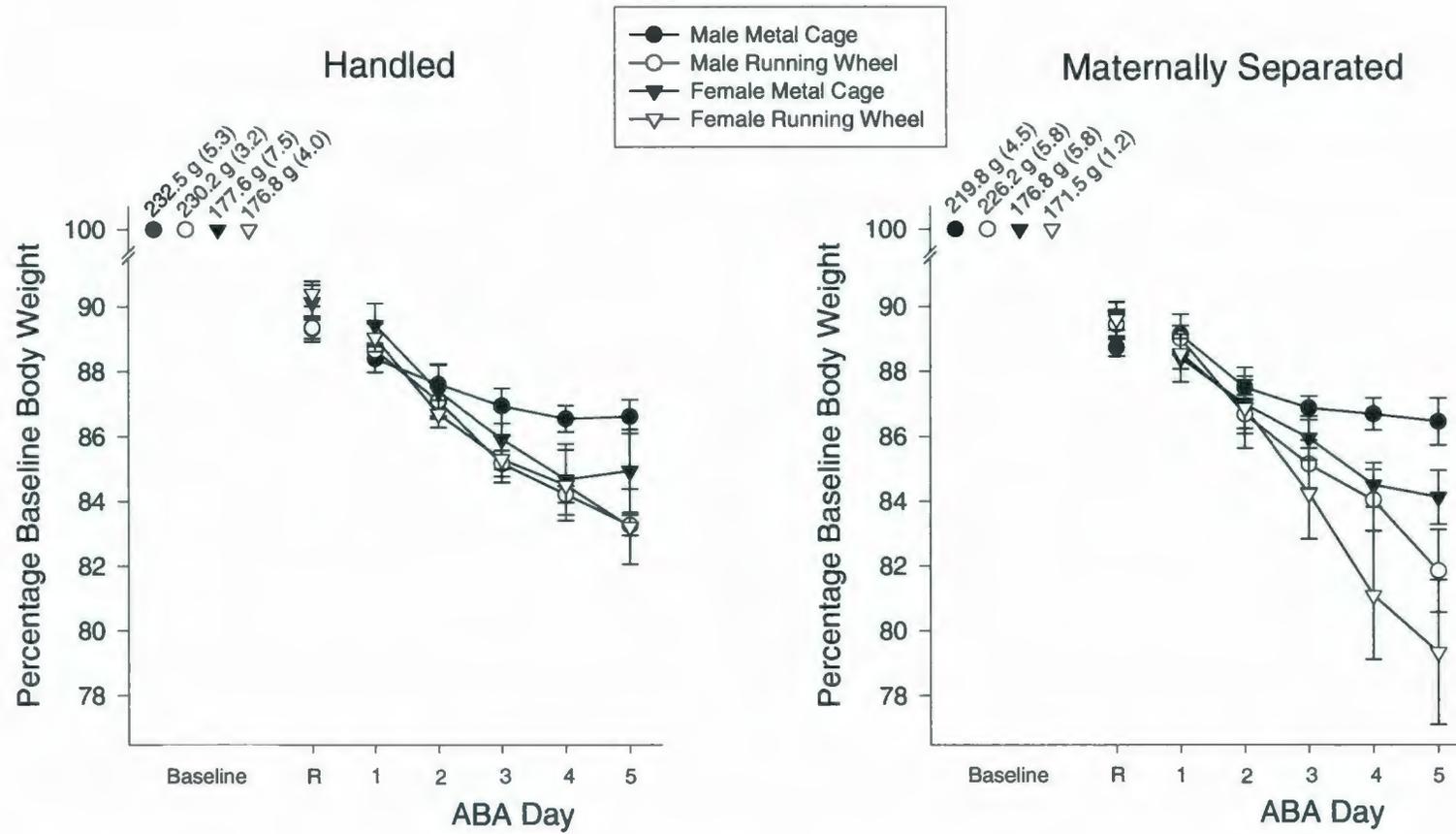


Figure 4.1. Adolescent rats' raw body weights during Baseline, and percentage of Baseline weights following 24 h of food Restriction (R) and during 5 days of ABA procedures. Data are presented separately for male and female animals that were handled (left) or maternally separated (right) during the postnatal period and assigned during the experiment to the metal cage or running wheel condition. Data points represent group means \pm standard error.

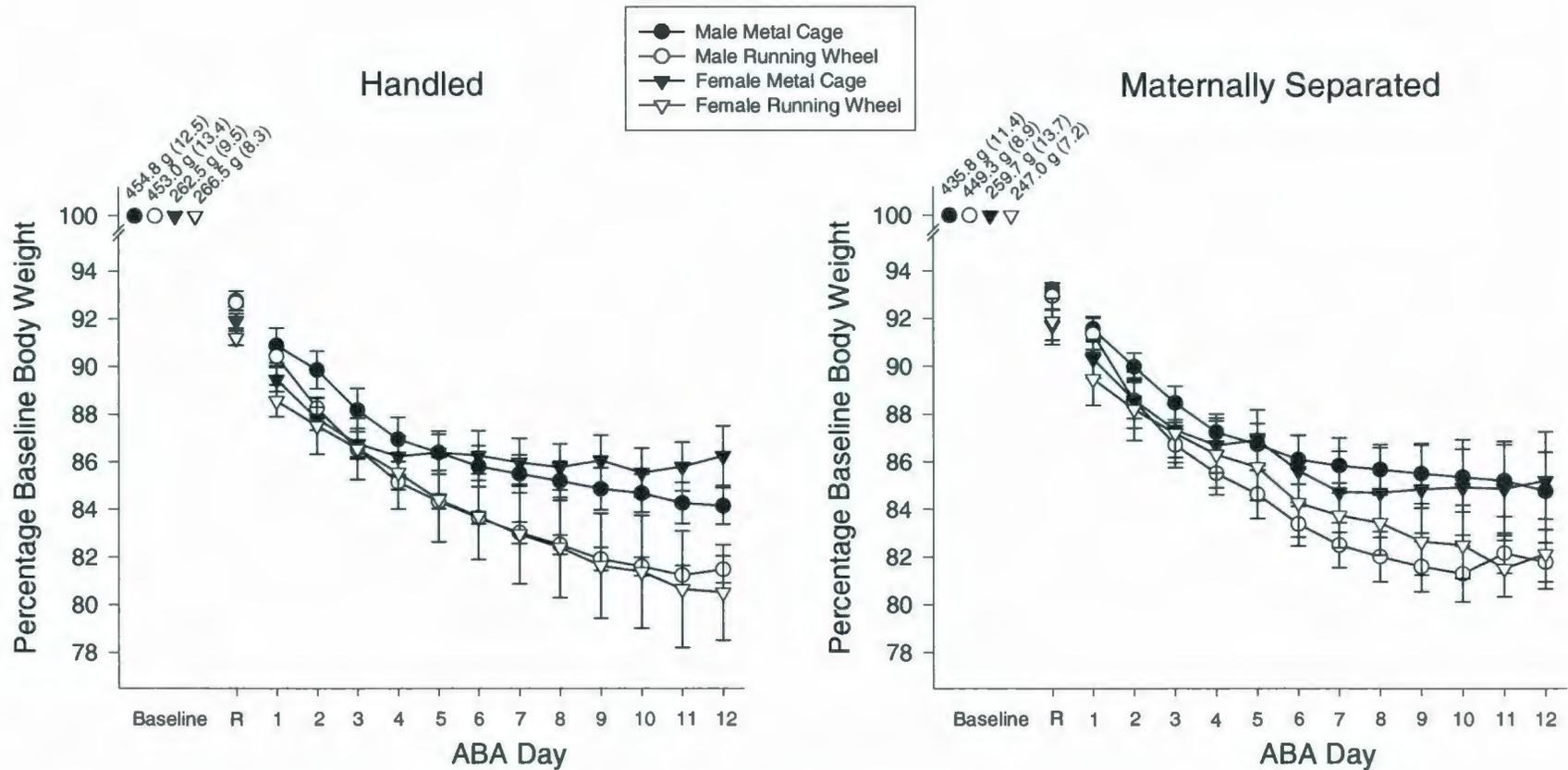


Figure 4.2. Adult rats' raw body weights during Baseline, and percentage of Baseline weights following 24 h of food Restriction (R) and during 12 days of ABA procedures. Data are presented separately for male and female animals that were handled (left) or maternally separated (right) during the postnatal period and assigned during the experiment to the metal cage or running wheel condition. Data points represent group means \pm standard error.

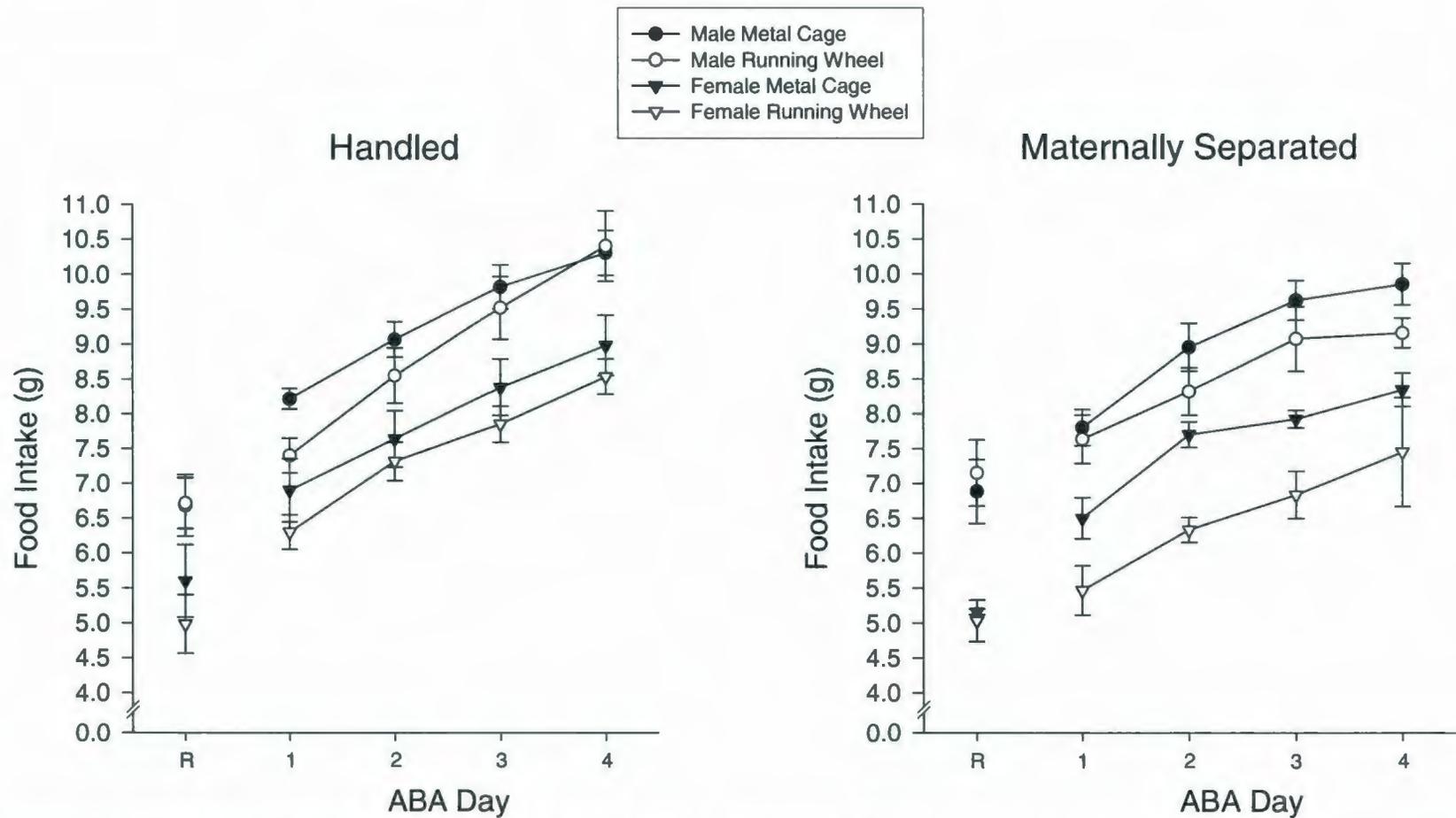


Figure 4.3. Daily food intake (g) of adolescent male and female, handled (left) and maternally separated (right) rats assigned to the metal cage or running wheel condition, following 24 h of food Restriction (R) and during the first 4 days of ABA procedures. Data points represent group means \pm standard error.

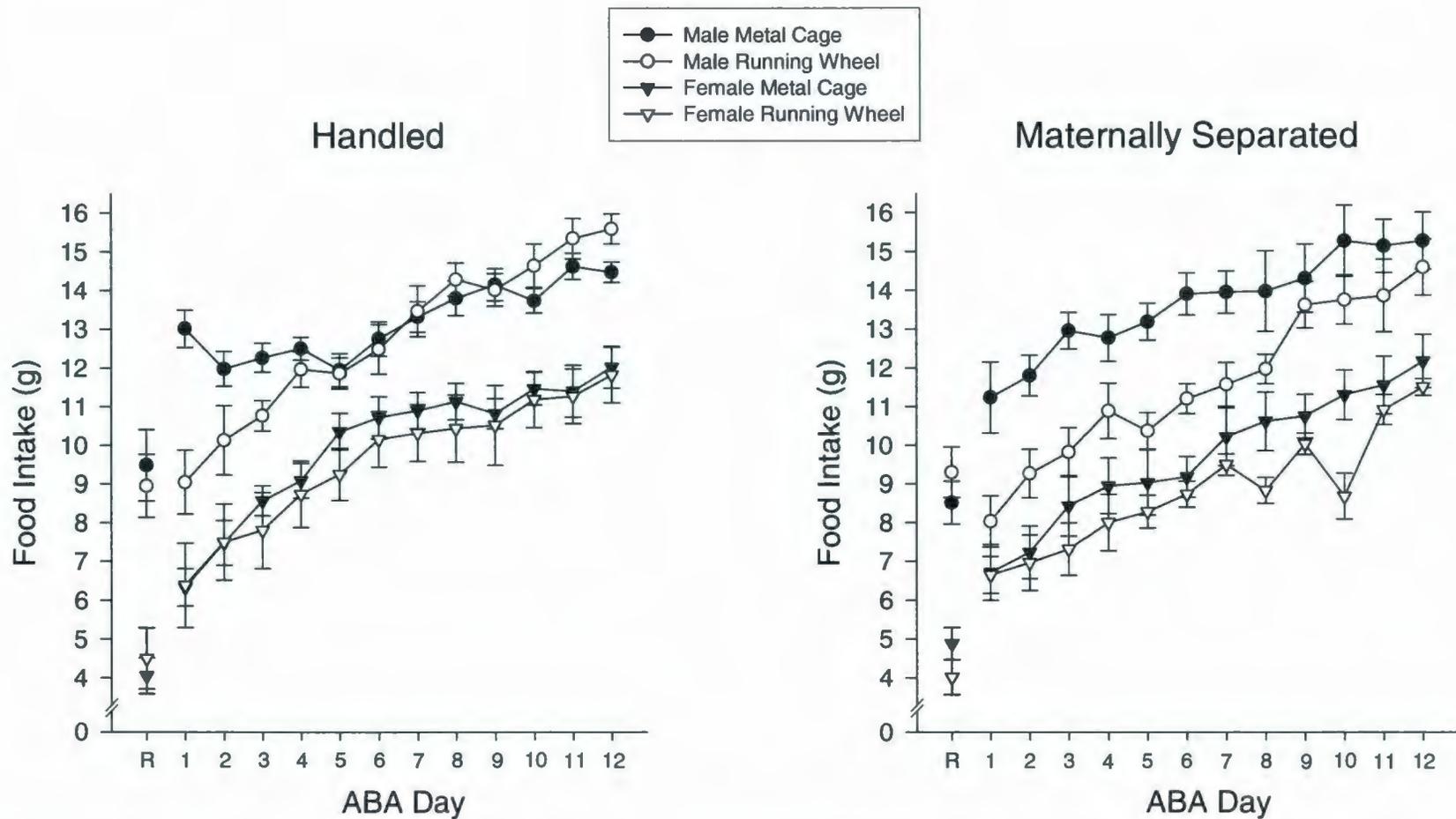


Figure 4.4. Daily food intake (g) of adult male and female, handled (left) and maternally separated (right) rats assigned to the metal cage or running wheel condition, following 24 h of food Restriction (R) and during the first 4 days of ABA procedures. Data points represent group means \pm standard error.

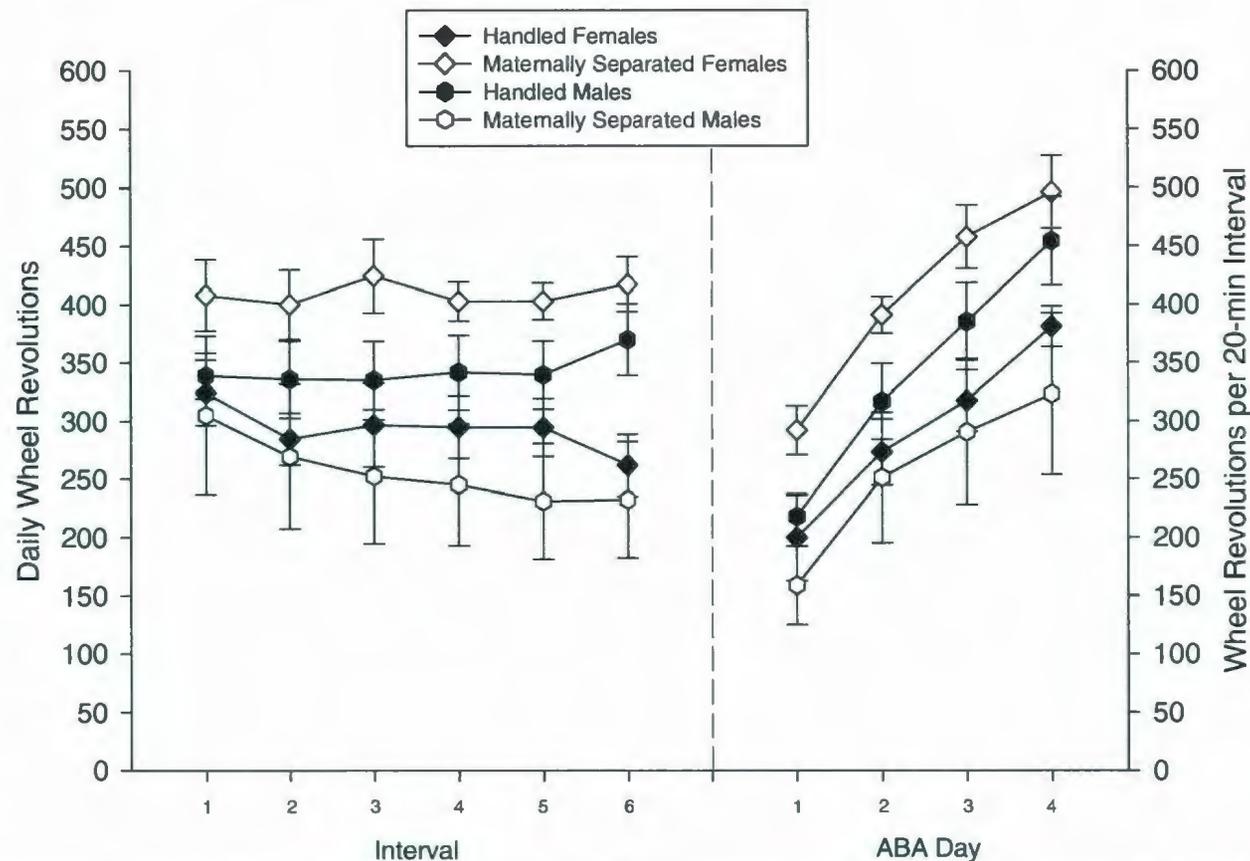


Figure 4.5. Adolescent male and female, handled and maternally separated rats' running rates during the first 4 of 12 ABA days. The left-hand side illustrates mean daily wheel revolutions for each of six 20-min intervals. The right-hand side portrays mean wheel revolutions per interval as a function of days. Cumulative daily wheel revolutions are equivalent to per-interval revolutions multiplied by 6. Data points represent group means \pm standard error.

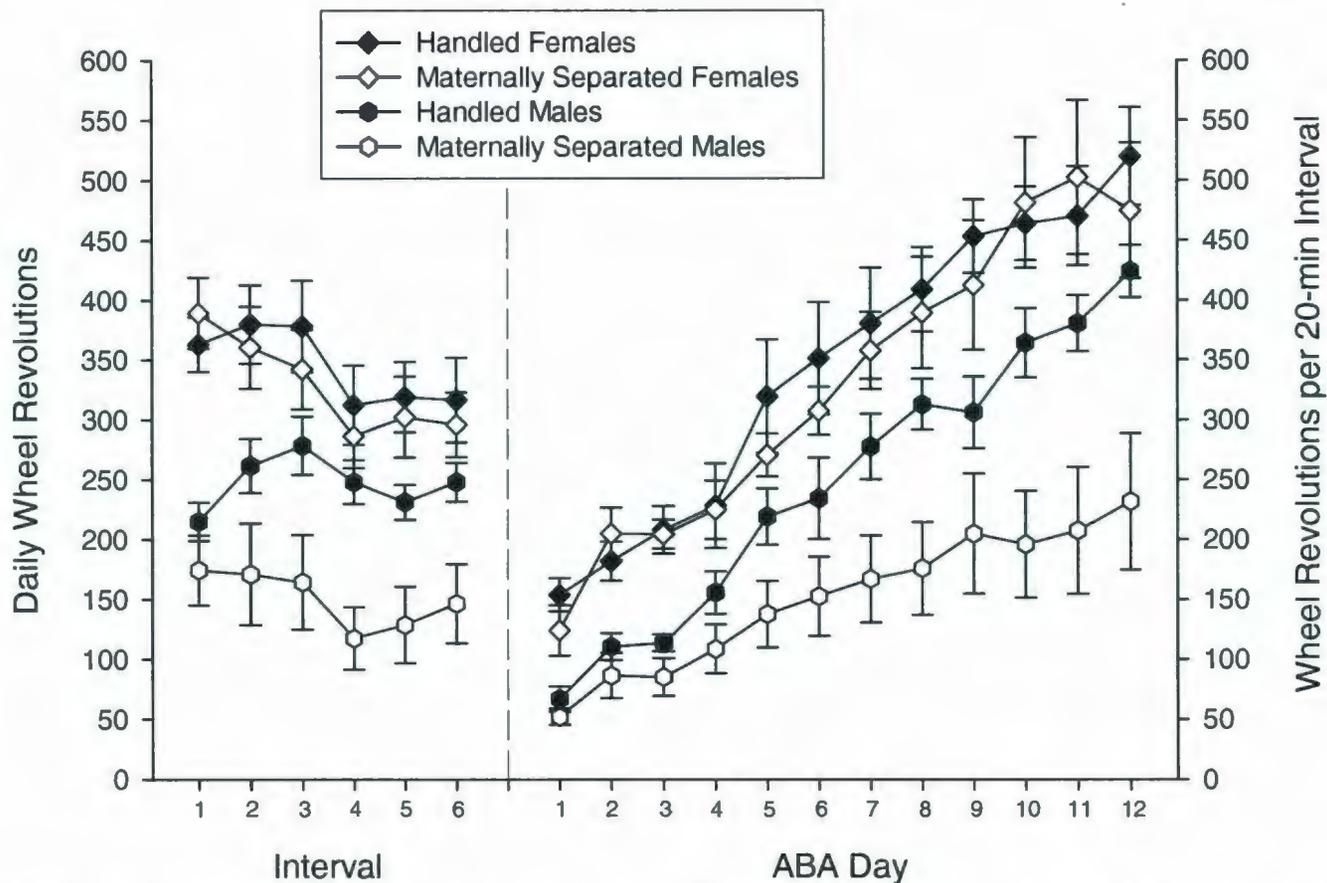


Figure 4.6. Adult male and female, handled and maternally separated rats' running rates during 12 ABA days. The left-hand side illustrates mean daily wheel revolutions at each of six 20-min intervals. The right-hand side portrays mean wheel revolutions per interval as a function of days. Cumulative daily wheel revolutions are equivalent to per-interval revolutions multiplied by 6. Data points represent group means \pm standard error.

Chapter 5: Summary and Future Research

Development of an eating disorder involves an interplay of factors, whether they be environmental, biological, developmental, genetic, and/or psychological. All forms of pathological eating, including binge eating disorder, bulimia nervosa, and both the restricting and binge/purge subtypes of anorexia nervosa, are more prevalent in females, have an increased propensity for adolescent onset (Hoek & van Hoeken, 2003), and are precipitated by stressful life events (Donohoe, 1984; Gluck, 2006; Kaye, 2008; Lo Sauro, Ravaldi, Cabras, Feravelli, & Ricca, 2008; Pyle, Mitchell, & Eckert, 1981; Swinbourne & Touyz, 2007; Wolff, Crosby, Roberts, & Wittrock, 2000). The value inherent in using an animal model to investigate the etiology of disordered eating and to test possible treatment options lies in the real-life validity of such a model. That is, animal models of eating disorders must attempt to reproduce the behavioural and physiological changes that characterize each disorder. Despite clinical evidence that early-life stress and heightened anxiety frequently precede the onset of pathological eating, animal models of eating disorders have not, for the most part, incorporated these findings. Nor do most models take into account that the largest population of eating-disordered individuals is comprised of adolescent females and young women.

The goals of this thesis were: (i) to present an overview of the physiological mechanisms that underlie stress-induced alterations in feeding systems and eating behaviours; and (ii) to examine potential factors that influence susceptibility to develop binge eating and anorexia using the most well-established animal models of these disorders. The research presented in Chapter 2 demonstrates that low levels of maternal

care in early life are associated with greater vulnerability to the later development of stress-induced binge eating of highly-palatable food and, further, that this heightened vulnerability manifests during adolescence. In Chapter 3, an activity-based animal model of anorexia nervosa (ABA) was used to examine the influence of early postnatal treatment on running-induced body weight loss and suppression of food intake. Young adult animals that experienced early-life maternal separation lost weight faster, ate less, ran more, and required fewer days to reach removal criterion compared to their handled counterparts, with females, in particular, showing increased vulnerability. These findings indicate that early postnatal treatment and sex influence the development of ABA. Finally, in Chapter 4, when a milder version of the ABA paradigm was used, early-life maternal separation increased females' susceptibility to ABA during adolescence, but not in adulthood, whereas males' susceptibility to ABA was increased only in adulthood. These results highlight the interplay between early postnatal treatment, sex, and developmental age in ABA etiology. Further, they are consistent with clinical evidence citing the relevance of early-life experience, sex, and developmental age to the development of anorexia nervosa (Connan, Campbell, Katzman, Lightman, & Treasure, 2003; Hoek & van Hoeken, 2003; Lo Sauro et al., 2008).

Notably, low levels of maternal licking and grooming or maternal separation in early life, each of which heightens stress reactivity, increased adolescent animals' propensity to develop stress-induced bingeing in an animal model of binge eating disorder, as well as stress-induced anorexia in the activity-based anorexia paradigm. The experimental design of each of these paradigms, specifically whether there is access to

highly-palatable food, likely played an important role in these findings. Recently, Brown, Avena, and Hoebel (2008) demonstrated that rats subjected to ABA procedures, comprised of unlimited wheel running and a 1-hr restricted feeding schedule, failed to exhibit running-induced loss of body weight when sweet, high-fat chow was made available during the daily meal. Consumption of high-fat, high-sugar foods increases opioid release which in turn inhibits HPA-axis activity (Dallman et al., 2003; Kreek & Koob, 1998; Yeomans & Gray, 2002), suggesting a mechanism by which sweet, high-fat food may blunt wheel-running-induced increases in HPA-axis activity. High-fat, high-sugar food was not available to wheel running animals during their once-daily meal in the current set of experiments (Chapters 3 and 4). Considering Brown et al.'s (2008) findings in combination with those reported herein, it would be interesting to test whether maternally separated animals might show increased consumption of high-fat, high-sugar food following wheel running should highly-palatable food be made available in an ABA paradigm. When animals subjected to restriction-refeeding-shock cycles were provided with high-fat, high-sugar peanut-butter chips, adolescent offspring of Low LG mothers demonstrated shock-induced bingeing on highly-palatable food, but not chow (Chapter 2). Had peanut-butter chips not been made available to animals in the binge eating protocol, one might speculate that no differences in food intake would have emerged between shocked and non-shocked rats. Indeed, shocked and non-shocked animals did not differ on chow intake on shock treatment day, although food-restricted, adolescent offspring of Low LG mothers did consume significantly *less* chow following footshock compared to their non-shocked counterparts in restriction-refeeding-shock cycle 3. These

findings suggest that, with their high stress reactivity, offspring of Low LG mothers subjected to repeated restriction-refeeding-shock cycles might have developed shock-induced anorexia had highly-palatable food not been available.

Animal models of eating disorders based on stressful events are promising in that they reproduce alterations in feeding behaviour and neuroendocrine function that are characteristic of each disorder. In addition to these alterations, there are other behavioural and cognitive changes that characterize eating-disordered individuals and that, for the most part, have not been examined in animal models. As mentioned previously, eating disorders are associated with altered affective states including heightened anxiety, restlessness, and agitation in anorexia nervosa (Casper, 1998; Garfinkel & Kaplan, 1986), and a high co-morbidity with mood and anxiety disorders in bulimia nervosa and binge eating disorder (Brewerton et al., 1995; Yanovski, Nelson, Dubbert, & Spitzer, 1993). Assessment of anxiety-like behaviours, such as number of entries and time spent in the open arms of an elevated plus maze or activity in an open field, and depressive-like behaviours, such as immobility in a forced-swim test, would substantially strengthen the face validity of an animal model if it reproduced affective changes prevalent in the clinical population. Cognitive dysfunction is an additional primary characteristic of most eating disorders. This includes preoccupation and compulsion, directed primarily towards food, so that individuals demonstrate exaggerated food obsessions, compulsive food restriction, and/or the inability to control food intake. Indeed, approximately 15% of anorexic patients and 21% of bulimic patients meet the diagnostic criteria for obsessive compulsive disorder (Halmi et al., 2005). Anorexic and binge eating individuals also

demonstrate deficits in attentional set-shifting. Given that tests of animal cognition, including obsessive-compulsive traits and attentional set-shifting, are already well-established (Eilam, Zor, Szechtman, & Hermesh, 2006; Robbins, 1998), this area of research could progress quickly. In the absence of large, prospective studies of eating disorders, it is difficult to determine whether these cognitive changes precede or follow the development of an eating disorder. Nonetheless, the fact that disrupted feeding patterns and cognitive changes co-occur in most eating disorders suggests that animal models should include assessments of both functions.

In addition to deficits in emotional attachment (Connan, Campbell, Katzman, Lightman, & Treasure, 2003; Ward, Ramsay, & Treasure, 2000), eating-disordered individuals frequently report a history of sexual and/or physical abuse during childhood, bullying and discrimination by peers, and/or other adverse life experiences (Jacobi, Hayward, de Zwaan, Kraemer, & Agras, 2004; Striegel-Moore, Dohm, Pike, Wilfley, Fairburn, 2002). As such, together with early postnatal treatment, other experimental manipulations that model early-life adversities experienced by humans, such as the chronic mild stress paradigm or severe sporadic stress (Pohl, Olmstead, Wynne-Edwards, Harkness, & Menard, 2007), might also result in varying susceptibility to the development of disordered eating.

Finally, the familial nature of eating disorders (Bulik, Sullivan, & Kendler, 2003; Bulik, Sullivan, Wade, & Kendler, 2000), suggests a genetic component. In humans, AgRP, 5-HT, estrogen, and cannabinoid receptor signaling have been implicated in anorexia, and MC4 receptor signaling in binge eating; animal models based on genetic

mutation or knock-out of these systems have been developed (see Hancock & Olmstead, in press, for a review). Selective breeding of animals has also been used to examine genetically-influenced behaviours and disordered eating. For example, mice selectively-bred for high levels of anxiety exhibit increased wheel-running rates and greater running-induced suppression of food intake and body weight in the ABA paradigm compared to mice selectively bred for low levels of anxiety (Gelegen et al., 2007). In general, however, most genetic-based animal models of eating disorders have limited applicability to humans, at least to date, as candidate genes for eating disorders are not well established (Klump & Gobrogge, 2005, Tozzi & Bulik, 2003). The next obvious step is to combine these experimental approaches to determine how genetic alterations and stressful events interact to produce maladaptive eating and physiological changes. Both processes clearly impact eating disorders in humans, a fact which should be reflected in animal models. With the development of increasingly-valid animal models of eating disorders that replicate the characteristics prevalent in the clinical population comes the potential, not only for treatment, but for the prevention of disordered eating.

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