

Activity-Based Anorexia in Adult Rats: A Behavioural and Neurobiological Investigation of
Resilience and Susceptibility

Stephanie Gallant

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This is to certify that the thesis prepared

By: Stephanie Gallant

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Signed by the final examining committee:

Dr. Peter John Darlington Chair

Dr. Nesha Burghardt External Examiner

Dr. Shimon Amir Examiner

Dr. Wayne Brake Examiner

Dr. Roisin O'Connor Examiner

Dr. Uri Shalev Supervisor

Approved by _____
Dr. Andreas Arvanitogiannis, Graduate Program Director

July 24, 2022 _____
Dr. Pascale Sicotte, Dean, Faculty of Arts and Science

ABSTRACT

Activity-Based Anorexia in Adult Rats: A Behavioural and Neurobiological Investigation of Resilience and Susceptibility

Stephanie Gallant, Ph.D.

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Anorexia nervosa (AN) is a life-threatening psychiatric illness characterized by severe caloric restriction, excessively low body weight, and extreme fear of weight gain. Up to 81% of individuals with AN exhibit high levels of physical activity which is associated with higher relapse, longer hospitalizations, and poorer outcomes. Mood and anxiety disorders, cognitive impairment, and alterations in reward processing are common in AN. Only about half of individuals with AN achieve full remission of symptoms and relapse is frequent. There is a pressing need for a better understanding of risk factors and underlying pathogenesis of AN. Activity-based anorexia (ABA) is an animal model of AN-like symptoms combining wheel access and food restriction resulting in increased running wheel activity, failure to increase food intake, and accelerated weight loss which can result in death if the animal is not removed from the experiment. While ABA has been used since the 1960s, only recently have researchers begun to explore the individual differences in response to ABA. The goal of this series was to establish the use of the model in our laboratory and to explore individual variability in ABA response by investigating behavioural and neurobiological differences between resilient and susceptible rats.

The experiments in Chapter 3 aimed to determine the ideal parameters to establish the use of the ABA model in our laboratory. ABA reliably developed in both male and female rats and female rats were particularly susceptible. Efforts to use behavioural measures of cognitive flexibility and anxiety- and depression-like behaviours during the ABA paradigm revealed that it is possible to do so without interfering with ABA development. In Chapter 4, we aimed to further characterize ABA susceptibility by assessing baseline anxiety- and depression-like behaviours, amphetamine-induced locomotor activity as an index of mesolimbic DA activity, and response to pharmacological treatment. In Chapter 5, we compared general neural activity

between resilient and susceptible rats in several key brain areas using c-Fos immunohistochemistry. We followed up on these findings by assessing response inhibition, a facet of impulsivity that is dependent on prefrontal functioning. In Chapter 6, we examined the effect of ABA on later cocaine-taking behaviours and assessed for trait differences in response to cocaine. Overall, by comparing resilient and susceptible rats, we found important differences in baseline running wheel activity as well as in performance on the forced swim task. We also observed statistical trends for differences in neural activity in the prefrontal cortex and nucleus accumbens as well as differential responding to cocaine self-administration. These results highlight the importance of considering susceptibility to ABA to further our understanding of risk factors involved in the development, maintenance, and treatment response in AN.

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CONTRIBUTION OF AUTHORS

Chapter 1: General Introduction

The general introduction was written by Stephanie Gallant and revised by Dr. Uri Shalev.

Chapter 2: General Methodology

The general methodology was written by Stephanie Gallant and revised by Dr. Uri Shalev.

Chapter 3: ABA development and its effect on measures of physiological and physiological functioning in Long-Evans male and female rats

Stephanie Gallant: Conceived and designed the experiments. Collected physiological and behavioural data. Conducted data analysis and interpretation and wrote the chapter.

Ashraf Mahmud: Helped with data collection.

Suzanne Trieu Chao: Helped with data collection.

Tracey D’Cunha: Helped with plasma collection.

Firas Sedki: Helped with running of ELISA and data interpretation.

Alexandra Usypchuk: Helped with set-shifting data collection in experiment 1.6.

Leon Mayers: Helped with set-shifting data collection in experiment 1.6.

Uri Shalev: Conceived and designed the experiments. Contributed to data analysis, interpretation of the results, and writing of the chapter.

Chapter 4: Resilience and susceptibility to ABA in female Sprague Dawley rats

Stephanie Gallant: Conceived and designed the experiments. Collected behavioural data.

Performed surgeries, prepared osmotic minipumps, completed drug preparation and administration. Conducted data analysis and interpretation and wrote the chapter.

Mei-Lissa Goudreau-Thibaudeau: Helped with surgeries, drug administration, and behavioural data collection. Contributed to data analysis and interpretation for experiment 2.3.

Alexandra Usypchuk: Helped with surgeries, drug administration, and behavioural data collection.

Michelle Da Paz: Helped with surgeries, drug administration, and behavioural data collection.

Nora Chujunian: Helped with surgeries, drug administration, and behavioural data collection.

Damaris Rizzo: Conducted surgeries for experiment 2.3.

Uri Shalev: Conceived and designed the experiments. Contributed to data analysis, interpretation of the results, and writing of the chapter.

Chapter 5: Differences in brain activity and impulsivity between ABA-resilient and ABA-susceptible rats

Stephanie Gallant: Conceived and designed the experiments. Collected behavioural data.

Collected and prepared brain tissue. Conducted fos immunohistochemistry. Conducted data analysis and interpretation and wrote the chapter.

Alexandra Usypchuk: Helped with behavioural data collection. Helped with brain tissue collection and fos immunohistochemistry. Conducted brain imaging and fos counting.

Michelle Da Paz: Helped with behavioural data collection. Helped with brain tissue collection and fos immunohistochemistry. Helped with brain imaging and fos counting.

Soraya le Noble: Helped with behavioural data collection. Helped with brain tissue collection and fos immunohistochemistry. Helped with brain imaging and fos counting.

Nora Chujunian: Helped with behavioural data collection. Helped with brain tissue collection and fos immunohistochemistry.

Mei-Lissa Goudreau-Thibaudeau: Helped with behavioural data collection.

Damaris Rizzo: Helped with behavioural data collection.

Firas Sedki: Helped conduct fos immunohistochemistry.

Uri Shalev: Conceived and designed the experiments. Contributed to data analysis, interpretation of the results, and writing of the chapter.

Chapter 6: Cocaine self-administration in ABA and its relation to ABA susceptibility

Stephanie Gallant: Conceived and designed the experiments. Collected behavioural data.

Assisted with surgeries. Completed drug preparation. Conducted data analysis and interpretation and wrote the chapter.

Michelle Da Paz: Conducted behavioural data collection. Assisted with surgeries. Contributed to data analysis and interpretation.

Damaris Rizzo: Performed surgeries. Helped with behavioural data collection.

Nora Chujunian: Assisted with surgeries. Helped with behavioural data collection.

Alexandra Chisholm: Performed surgeries.

Uri Shalev: Conceived and designed the experiments. Contributed to data analysis, interpretation of the results, and writing of the chapter.

Chapter 7: General Discussion

The general discussion was written by Stephanie Gallant and revised by Dr. Uri Shalev.

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ABBREVIATIONS

ABA	activity-based anorexia
AN	anorexia nervosa
AN-BP	anorexia nervosa binge-purge type
AN-R	anorexia nervosa restrictive type
AMPH	amphetamine
ANOVA	analysis of variance
BN	bulimia nervosa
BLA	basolateral amygdala
CA	central amygdala
CgL	cingulate cortex
CLZ	clozapine
CNO	clozapine-N-oxide
CSF	cerebrospinal fluid
DA	dopamine
DAT	dopamine transporter
dIPFC	dorsolateral prefrontal cortex
DREADD	designer receptors exclusively activated by designer drugs
DSM-5	diagnostic and statistical manual of mental disorders - 5
D1/D1R	dopamine-1 receptor
D2/D2R	dopamine-2 receptor
ELISA	enzyme-linked immunosorbent assay
EPM	elevated plus maze
FA	feeding activity
FAA	food anticipatory activity
FR	food restricted
FST	forced swim test
GABA	gamma-aminobutyric acid
GIC	granular insular cortex
GHS-R1a	growth hormone secretagogue receptor
HPA	hypothalamic-pituitary-adrenal

ILC	infralimbic cortex
IR	Immunoreactive/immunoreactivity
LH	lateral hypothalamus
MANOVA	multivariate analysis of variance
MDD	major depressive disorder
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
NA	nocturnal activity
NAcc	nucleus accumbens
OCD	obsessive-compulsive disorder
OLZ	olanzapine
PD	postnatal day
PET	positron emission tomography
PFC	prefrontal cortex
PPA	posprandial activity
PrL	prelimbic cortex
SPSS	Statistical package for the social sciences
SPT	sucrose preference test
TBS	Tris-buffered saline
VEH	vehicle
VTA	ventral tegmental area
WCST	wisconsin card sorting task
ZT	zeitgeber time

CHAPTER 1: GENERAL INTRODUCTION

Anorexia nervosa (AN) is a life-threatening psychiatric illness with a lifetime prevalence of 0.9% (Keski-Rahkonen et al., 2007). The hallmark of AN is excessively low body weight achieved through extreme food restriction and/or persistent behaviours interfering with weight gain such as physical activity. Individuals with AN also experience intense fear of weight gain and disturbances in body image which maintain the pattern of self-starvation. AN affects mostly young women and the age of onset has been shown to be decreasing in younger generations (Favaro, Caregaro, Tenconi, Bosello, & Santonastaso, 2009). Due to its associated medical complications, AN is the deadliest of all psychiatric illnesses with a mortality rate ranging from 5 to 10% (Arcelus, Mitchell, Wales, & Nielsen, 2011). Furthermore, up to 30% of individuals with AN attempt suicide during the course of illness and completed suicide accounts for 1 in 5 deaths in individuals with AN (Arcelus et al., 2011). Treatment of AN, typically consisting of a combination of psychotherapy, nutritional rehabilitation, and pharmacotherapy, is a challenge and relapse rates remain high, ranging from 35% to 41% within 12 months post-treatment (Carter, Blackmore, Sutandar-Pinnock, & Woodside, 2004; Carter et al., 2012; McFarlane, Olmsted, & Trottier, 2008). It is estimated that only half of individuals with AN achieve full remission of symptoms, and even recovered individuals typically maintain a low weight (Steinhausen, 2002). Clearly, there is a pressing need for more efficacious treatment options which necessitates a better understanding of the underlying pathogenesis of AN. Leading eating disorder researchers are now recommending that future research focus on the identification of risk factors and other preventative strategies (DeSocio, O'Toole, Nemirow, Lukach, & Magee, 2007). More basic research is needed to deepen our understanding of the illness and animal models are an essential research tool. The first goal of the work presented in this dissertation was thus to test the reliability of, and to further validate the leading animal model of AN-like symptoms, activity-based anorexia (ABA) and to ultimately use this model to expand on the neurobiological understanding of this devastating disorder. A second crucial goal was to investigate the differences between animals that are more resilient and those that are more susceptible to the model in hopes of shedding light onto factors that may increase the risk of AN in humans.

Anorexia Nervosa

Diagnostic and Statistical Manual of Mental Disorders (DSM-5)'s criteria

The DSM-5 describes three criteria that must be met for a diagnosis of AN to apply: A) restriction of energy intake resulting in significantly low body weight (i.e., a weight that is lower than expected given a person's age and sex); B) fear of gaining weight or of becoming fat or behaviours interfering with weight gain; and C) distortion in perception of body weight or shape, excessive influence of body weight or shape on self-evaluation, or lack of recognition of the severity of the low body weight. The DSM-5 further divides the diagnosis of AN into two subtypes: 1) AN Restricting type in which weight loss is accomplished through dieting, fasting, and/or excessive exercise without presence of binge eating or purging behaviour (vomiting, laxatives, diuretics, or enemas) or 2) AN Binge eating/purging type in which recurrent episodes of binge eating or purging behaviours are present (American Psychiatric Association, 2013). Beyond the DSM-5 depiction of AN, there are many additional features of AN that are not necessary nor sufficient for diagnosis, but that are nonetheless common and that must be taken into consideration in the development or evaluation of animal models of AN-like symptoms. Such features include behavioural symptoms, personality traits, neurocognitive processes, clinical comorbidities, as well as physical and neurobiological features.

Behavioural features of AN

Food restriction and binging. Restriction of energy intake is one of the defining characteristics of AN and can take different forms. Individuals with AN may restrict their energy intake by abstaining from all foods for days at a time, skipping meals, eating reduced portion sizes at fixed mealtimes, or simply consuming low caloric foods (Elran-Barak et al., 2015). While some individuals with AN never engage in episodes of binge eating (uncontrollable eating), there is a subset of patients who do engage in binge eating which may or may not be accompanied by compensatory purging. Unlike bulimia nervosa (BN), however, in which under-eating and over-eating cancel each other out resulting in unremarkable body weight, under-eating predominates in AN resulting in severe weight loss (Fairburn, Cooper, & Shafran, 2003).

Compulsive body checking. In AN individuals who over evaluate the importance of their body shape and size (and their ability to control them), body checking behaviours are also present and implicated in the maintenance of both shape and weight concerns and dietary restriction (Fairburn et al., 2003). Body checking behaviours include, but are not limited to,

checking ones reflection in the mirror, glass doors, etc., pinching body parts to measure fat, rubbing skin to check for fat and cellulite, measuring body parts (e.g., wrist diameter), trying on clothes to see if they still fit, reassurance-seeking (e.g., “do I look fat?”), and asking others about their weight and clothing size for comparison (Reas, Whisenhunt, Netemeyer, & Williamson, 2002). Such checking behaviours can be viewed as being compulsive in nature in that they serve to reduce distress stemming from body size and shape concerns (Rosen, Srebnik, Saltzberg, & Wendt, 1991).

Purging behaviours. In addition to dietary restriction, bingeing, and compulsive body checking, some individuals engage in various forms of behaviours that interfere with weight gain. Purging behaviours include self-induced vomiting and misuse of laxatives, diuretics, or enemas. While purging behaviours typically follow an episode of binge eating, some individuals with AN do not binge but do regularly purge after consuming small amounts of food (DSM-5; American Psychiatric Association, 2013). Excessive exercise is another common form of weight control and, in one study, was present in 80.8% of AN patients during the acute phase of illness (Davis et al., 1997). The DSM-5 defines exercise as being excessive when it interferes with important activities, occurs in inappropriate times or settings, or when exercise is continued despite medical complications.

Hyperactivity. Another important feature of AN and one that is of particular interest for the ABA model and the present dissertation is “hyperactivity” which includes, but is not limited to, excessive exercise. Clinicians have long been fascinated by the seemingly paradoxical elevated levels of physical activity in a subset of individuals with AN (Gull, 1888). This elevated level of physical activity (hereafter referred to as “hyperactivity”) has also been described as “overactivity”, “motor restlessness”, “diffuse restlessness”, “paradoxical liveliness”, and “abundance of physical energy” (Hebebrand et al., 2003). In addition to the wide range of descriptors used, various measurement techniques have also been used to quantify hyperactivity in AN such as retrospective analysis of medical records, activity diaries, assessment through semi-structured interviews, expert and self-report rating scales of physical activity and motor restlessness, and physical measurements using pedometers or accelerometers (Hebebrand et al., 2003). Prevalence rates of hyperactivity among individuals with AN range from 31-80% (Casper, 2006; Davis, Kennedy, Ravelski, & Dionne, 1994; Dittmer, Jacobi, & Voderholzer,

2018; Hebebrand et al., 2003; Rizk, Lalanne, Berthoz, Kern, & Godart, 2015), a range that is large but unsurprising given the variety in operational definitions and measurement techniques used. The various definitions and measurements of hyperactivity reflect different aspects of a related behaviour and allude to the uncertainty about the origin, function, and maintaining factors of hyperactivity in AN. Indeed, while the concept of hyperactivity in AN does include excessive exercise as a weight loss strategy, it also includes compulsive exercise and general excessive motor movements and restlessness that may be explained by more complex mechanisms. Many of such hypothesized mechanisms come from animal studies and include activation of dopaminergic reinforcing pathways that would render the behaviour as rewarding (Park, Godier, & Cowdrey, 2014a), low levels of the fat-derived hormone leptin (Hebebrand et al., 2003), hyperactivity as a thermoregulatory behaviour to prevent hypothermia (Carrera et al., 2012), or the interpretation of hyperactivity as a form of foraging behaviour (Cornish & Mrosovsky, 1965). Regardless of the underlying mechanism or function of the behaviour, hyperactivity, relative to the degree of dietary restriction in individuals with AN, is considered a fundamental clinical feature of the disorder. Furthermore, this feature complicates recovery as it is difficult to control during treatment and interferes with weight gain. Hyperactivity in AN is also associated with higher relapse rates following treatment, longer hospitalizations, and poorer prognosis (Davis et al., 1997).

Personality traits associated with AN

Personality traits reflect people's characteristic patterns of thoughts, feelings, and behaviours that are relatively stable across different situations. In the effort to identify predisposing factors of AN, personality traits have received a lot of attention in eating disorder research. Examples of traits that have emerged as being common in individuals with AN include negative emotionality/neuroticism, perfectionism, impulsivity/negative urgency, harm avoidance, and reward dependence. Negative emotionality, perfectionism, and negative urgency have received particular attention as they have been shown to prospectively predict the development of eating disorder symptoms (Culbert, Racine, & Klump, 2015). Negative emotionality refers to the disposition towards experiencing unpleasant emotions such as anxiety and anger and the trait has been shown to predict the development of eating pathology such as purging behaviours and the drive for thinness (Leon, Fulkerson, Perry, Keel, & Klump, 1999). Perfectionism, the holding

of high personal standards and overly critical self-evaluations, is another trait that has been repeatedly associated with AN. Results from longitudinal studies suggest that perfectionism is a risk factor of AN by acting either independently or in combination with other important factors such as low self-esteem or body dissatisfaction (Culbert et al., 2015). The trait of impulsivity has also been associated with eating disorder pathology and, in the context of AN, appears to be specifically related to those individuals who engage in binge and purging behaviours. Earlier studies found that behavioural indices of impulsivity, such as substance use and delinquency, predicted the onset of binge eating and compensatory behaviours in adolescent females (Wonderlich, Connolly, & Stice, 2004). Impulsivity is a multifaceted construct and efforts to deconstruct it have resulted in the identification of negative urgency – the tendency to engage in rash action when distressed – as the most important form of impulsivity in the prediction of binge and purging (Fischer, Peterson, & McCarthy, 2013).

Neurocognitive processes in AN

Impulsivity. Neurocognitive processes have increasingly been examined in eating disorders in an effort to identify risk factors. As mentioned above, impulsivity has been associated with eating disorders and represents a complex and multifaceted construct. Inhibitory control is one of many facets of impulsivity and refers to the suppression, inactivation, or overriding of a relatively automatic response in favor of a less automatic one (Culbert et al., 2015). Deficits in inhibitory control have been reported in individuals with AN, particularly in the binge/purge subtype, using a Go/No-Go task (Claes, Vandereycken, & Vertommen, 2005; Rosval et al., 2006). Furthermore, neuroimaging studies of inhibitory control in AN have demonstrated reduced activation in frontostriatal regions compared to control participants (Culbert et al., 2015). Impulsivity and inhibitory control are further discussed in Chapter 5 (experiment 3.2).

Compulsivity. Compulsivity is another neurocognitive construct that has been studied in the context of AN and broadly refers to repetitive behaviours that are performed according to certain rules or in a stereotypical fashion and that serve the function of relieving anxiety (Godier & Park, 2014; Robbins, Gillan, Smith, Wit, & Ersche, 2012). Similar to impulsivity, compulsivity is a multidimensional construct that can be further broken down into domains that can be assessed through different neurocognitive tasks. Cognitive flexibility is one such domain

that has been particularly relevant in AN and refers to the ability to “shift” between multiple tasks, operations, or mental sets (Roberts, Tchanturia, Stahl, Southgate, & Treasure, 2007). Performance on cognitive flexibility tasks has repeatedly been shown to be impaired in adults with AN compared to healthy controls (Wu et al., 2014). There is also evidence suggesting that such deficits persist into recovery from AN (Lindner, Fichter, & Quadflieg, 2014; Roberts, Tchanturia, & Treasure, 2010; Shott et al., 2012). Cognitive inflexibility in AN has been associated with perfectionism, rigid thinking, perseverative actions to control body weight and shape, increased anxiety and depression, and higher mortality rate (Roberts et al., 2010). Compulsivity, and more specifically cognitive flexibility, are further discussed in Chapter 3 (experiment 1.6).

Clinical comorbidity

Given the discussed psychological features that are commonly observed in AN, it should be of no surprise that individuals with this eating disorder often present with additional psychiatric comorbidities. In fact, comorbidity rates have been shown to be as high as 73% in individuals with AN restriction type and 82% in AN binge/purge type (Herzog, Nussbaum, & Marmor, 1996). Of all psychiatric diagnoses, mood disorders, anxiety disorders, obsessive-compulsive disorder (OCD), and substance use disorders are the most likely to co-occur with AN (Salbach-Andrae et al., 2008)

Mood disorders. Mood disorders are particularly prevalent in AN with rates reaching as high as 89% (Fornari et al., 1992). In one study examining the prevalence of mood disorders in a sample of individuals with AN, the authors determined that mood disorders were significantly more frequent in individuals with AN compared to controls and mainly consisted of major depressive disorder (MDD; Godart et al., 2015). In their sample of individuals with AN, Godart et al. (2015) found the prevalence of MDD to be of 63.9% in the restrictive subgroup and 74.5% in the binge/purge subgroup. The chronology of onset of MDD in individuals with AN varied greatly suggesting that mood disorders may be a risk factor for the development of AN, a result of malnutrition during acute AN, and/or a long-term consequence of AN.

Anxiety disorders. Anxiety disorders, particularly social anxiety disorder and generalized anxiety disorder, are also common comorbidities in AN. In a sample of women presenting for treatment for an eating disorder, (AN or BN), 65% met diagnostic criteria for at least one

comorbid anxiety disorder with social anxiety disorder being the most frequent (42%) followed by generalized anxiety disorder (23%), panic (3%), and specific phobia (2%; Swinbourne et al., 2012). Importantly, of the women presenting with comorbid anxiety, 69% reported the onset of the anxiety disorder to have preceded the onset of their eating disorder (Swinbourne et al., 2012). These results are consistent with earlier reports by Kaye, Bulik, Thornton, Barbarich, and Masters (2004) that at least two-thirds of individuals with an eating disorder had one or more lifetime anxiety disorders, the majority of which developed during childhood, prior to the onset of the eating disorder. These findings support the possibility that anxiety is a vulnerability factor for the development of eating disorders.

OCD. The compulsive behaviours observed in AN have often been compared to those of OCD where, in AN, the obsession focus is on eating, weight, and shape (Godier & Park, 2014). As in OCD, individuals with AN have persistent intrusive thoughts regarding food and weight gain, and may develop compulsive behaviours in an effort to neutralize the anxiety associated with these thoughts (Steinglass & Walsh, 2006). Kaye et al. (2004) reported that in their sample of individuals with AN, approximately 40% also met diagnostic criteria for OCD.

Substance use disorders. Finally, substance use disorders have also been shown to be prevalent in individuals with AN with lifetime prevalence rates varying largely between 6% to 55% (Salbach-Andrae et al., 2008). The relationship between substance use disorder and AN is one of the most intensely studied areas in eating disorder clinical research. In a large population-based sample, eating disorders were associated with greater substance use relative to the nonclinical control group (Root et al., 2010). More specifically, Root et al. (2010) found that individuals with AN had significantly increased odds, relative to the nonclinical control group, of abusing alcohol, diet pills, cannabis, and illicit drugs such as hallucinogens, opioids, sedatives, and stimulants. Substance use in eating disorders, and in AN specifically, is further discussed in Chapter 6.

Physical and neurobiological features of AN

Physical effects of AN. Extreme dietary restriction produces a wide range of physical symptoms as the body struggles to function under conditions of insufficient nutrients and calories. Some of the physical effects of malnutrition that are commonly observed in individuals with AN include constipation, muscle weakness, poor circulation, bloating, delayed gastric

emptying, dizziness, abdominal pain, and skin dryness. These symptoms typically return to normal after weight restoration (Fairburn, 2008).

There are, however, longer-term physical effects of dietary restriction that are often not completely reversible. One such complication is amenorrhea. Up to 78% of women with AN have amenorrhea which may impact fertility if the menstrual cycles and ovulation are suppressed for long periods of time (Pinheiro et al., 2007). Other long-term consequences of extreme dietary restriction include osteoporosis, blood conditions such as anaemia or pancytopenia which is a life-threatening condition resulting from a reduction of blood cell production by the bone marrow, and cardiovascular complications such as arrhythmias, bradycardia, and hypotension (Mehler & Brown, 2015).

Neurobiological alterations. AN is also associated with alterations in neurobiological functioning. These alterations are typically present premorbidly, exaggerated by malnutrition, and return to premorbid levels after recovery. Thus, such neurobiological features offer promising lines of research in efforts to identify risk factors and preventative strategies. Neurobiological changes include, but are not limited to, alterations in neuronal systems, regional cerebral blood flow, glucose metabolism, and pubertal hormones. A review of all these changes are beyond the scope of this dissertation but are documented in recent reviews by Higgins (2019) and Phillipou, Rossell, and Castle (2014).

Dopamine (DA). Several neurotransmitters have been shown to be impacted in AN and include DA, serotonin, and norepinephrine. Though a review of all neurotransmitter systems impacted in AN is beyond the scope of this dissertation (reviewed in Higgins, 2019), changes in DA merit attention in the context of the experiments presented here. Individuals with AN often present as anhedonic and often tend to engage in obsessive or ritualistic behaviours, such as food rituals, that have been linked to disruptions in dopaminergic functioning (Davis & Woodside, 2002; Reas et al., 2002). Given DA's central role in reward processing of both drug and natural rewards (such as food) and motivation, it seems intuitive that dopaminergic functioning would be impaired in AN. Indeed, several lines of evidence suggest that individuals with AN have altered dopaminergic functioning, though the direction of change is unclear. Based on the observation that drugs that increase DA (e.g., amphetamine) lead to AN-like symptoms, Barry and Klawans (1976) initially proposed that increased DA plays a central role in the illness. Subsequent studies

examining DA in AN, however, have reported increased, decreased, or unchanged levels in AN (Kontis & Theochari, 2012). The current state of research on DA in AN is further discussed in Chapter 4.

Structural and neural changes. Various neuroimaging studies show substantial structural and neural abnormalities in the brain among individuals with AN though uncertainty remains about whether these anomalies constitute premorbid traits or are consequences of malnutrition. Decreased volumes of white and gray brain matter have been documented in the cerebellum, hypothalamus, caudate nucleus, and frontal, parietal, and temporal areas (Boghi et al., 2011; Higgins, 2019) as well as in the cingulate cortex (Friederich et al., 2012) in acute phases of AN. These results are consistent with earlier reports of enlarged ventricles in individuals with AN (Kaye, 2008). This brain atrophy, however, has been suggested to be the result of malnutrition and cerebral dehydration (Boghi et al., 2011) and has been shown to normalize following recovery (Seitz et al., 2014). In contrast, anomalies in brain blood flow and activity in individuals with AN appear to persist after recovery. For instance, reduced blood flow was observed in the frontal, parietal, temporal, and occipital areas of the brain of weight-restored individuals with AN 7 years after the onset of illness (Råstam et al., 2001). When exposed to pictures of food or to the taste of food, both individuals with AN and those who had recovered from AN showed overactivation of the frontal and anterior cingulate cortex and insula compared to healthy controls (Cowdrey, Park, Harmer, & McCabe, 2011; Santel, Baving, Krauel, Münte, & Rotte, 2006).

Animal models of AN

Animal models have been a powerful tool in research on neuropsychiatric conditions. Etiological understanding of the condition in question usually provides a good basis for developing an appropriate animal model. For instance, if potential risk genes for a disease in humans have been identified, the homologous genes in animal models can be mutated or deleted (Kim, 2012). In cases where causes of illness have not been determined, understanding the progression of the illness and its treatment may provide useful information in developing a relevant animal model. Unfortunately, the limited information on the causes, development, and treatment of AN has hampered the development and use of animal models of AN. Additionally, due to the complexity of AN, researchers have had to turn towards models that mimic only a few

of the many clinical features of AN. Despite these challenges, several animal models of AN have been proposed, each with its strengths and weaknesses. Such models include, but are not restricted to, stress-induced anorexia, separation models, diet restriction models, and ABA which is the model used in the present dissertation. For a recent and thorough review of existing animal models of AN-like behaviours, see Scharner and Stengel, (2021).

Stress-induced anorexia

It has been reported that life stressors resulting in imbalances in the hypothalamic-pituitary-adrenal (HPA) axis are common in eating disorders (Kim, 2012). Indeed, stress-mediated changes in the HPA axis can affect food intake (Jahng, 2011; Lo Sauro, Ravaldi, Cabras, Faravelli, & Ricca, 2008). Therefore, stress models have frequently been used to model loss of appetite in rodents. Several variations of stress models exist including cold water swimming, tail pinching, physical restraint, and direct brain stimulation, all of which have been shown to result in loss of appetite and weight loss in rodents (Shimizu, Oomura, & Kai, 1989; Wilson & Cantor, 1986). One advantage of stress models is that they produce weight loss and reduced food intake without the need to manipulate food availability. However, caution is required when implementing such protocols to avoid causing unnecessary harm to the animals. Furthermore, it has been suggested that stress models are not suited to mimic AN because they are based on the erroneous assumption of appetite loss in humans with AN (Casper, Sullivan, & Tecott, 2008). Another limitation of stress protocols as models of AN is that the stressors used (e.g., restraint) are not symptoms of AN (Casper et al., 2008). Rather, stress models may be more relevant as models of risk factors for AN.

Separation model

To mitigate the risk of physical harm to animals that is present in acute stress models, models employing milder and more chronic forms of stress have been proposed. An example of this is the separation model in which rodents are housed in one cage but separated by Plexiglass partitions which allows them to see and smell each other without having direct contact. In this model, separation acts as a chronic stressor to induce a depression-like condition with decreased feeding, weight loss, and impaired cognitive functioning (Casper et al., 2008). While the chronic stress caused by physical isolation may be more relevant to the human condition than acute stressors, it may also be more relevant in the study of factors increasing AN risk.

Diet restriction models

Excessive diet restriction alone has also been used as a model of AN in rodents and unsurprisingly results in weight loss. Diet restriction in this context typically takes one of two forms – either a certain amount of food, calculated as a percentage of *ad libitum* food intake, is available to the animal continuously or unlimited food is provided continuously for a predetermined duration (e.g., 1.5 h/day). Diet restriction in rodents has been shown to mimic many of the changes in neuroendocrine and cognitive functioning observed in AN (see Kim, 2012). A major criticism of this model, however, is that food intake is being limited by the experimenter and not by the animal.

Activity-based anorexia

Finally, ABA is one model that has gained significant traction since its origin in the 1960s and it addresses some of the limitations of the previously discussed models. More specifically, unlike the stress models using acute stressors such as restraint or tail pinching, the ABA model involves manipulations that are relevant to the clinical manifestation of AN, namely hyperactivity and dietary restriction. Aside from the potentially dangerous weight loss that is inevitable in all models of AN, there are no additional risks of physical harm to the animals in ABA. Like in diet restriction models, food intake in ABA is manipulated and initially limited by the experimenter. It can be argued, however, that although the initial caloric intake is under the control of the experimenter, a self-restriction eventually develops as the animal becomes more active.

Routtenberg and Koznesof, traditionally credited with the development of the ABA model, were originally investigating the broad relation between reward and nonadaptive behaviours. Earlier works had identified marked weight loss in rats with continued access to a running wheel (Spear & Hill, 1962) and the inability of rats on a food restriction schedule to maintain stable body weight (Hall & Hanford, 1954; Weasner, Finger, & Reid, 1960). Routtenberg and Kuznesof (1967) built on these findings by conducting a series of experiments examining the effects of various food restriction schedules on active (housed in cages equipped with running wheels) and inactive male rats. They found that active rats, compared to the inactive controls, had lower food intake, were unable to compensate for energy expenditure during activity, and could even starve themselves to death if the experiment was not stopped. On

the contrary, inactive control rats on the same food restriction schedule were able to stabilize their body weight, indicating the important role of activity in this model. This phenomenon became known as ABA and has since been used under various protocols as a model of AN. Although there is currently significant heterogeneity in ABA protocols, designs typically consist of a 1-2 h feeding period, usually during the beginning of the dark phase, combined with continuous access to a running wheel. Most recent ABA protocols also include a wheel acclimatization period prior to the start of the food restriction. Furthermore, the ABA protocol is usually continued until a certain weight loss criterion (i.e., starvation criterion) is reached, commonly defined as between 15 and 30% weight loss (Schalla & Stengel, 2019). Since its inception, the ABA model has been shown to mimic key behavioural, biological, and psychological features of AN.

Behavioural effects of ABA. While some of the behavioural features of AN are impossible to replicate in rodents (e.g., body checking and purging), ABA is the only model that captures the hyperactivity that is observed in many individuals with AN. Indeed, following a period of stable activity (in protocols using a wheel acclimatization phase), the introduction of food restriction has been consistently shown to result in significantly increased running wheel activity. The intensity of this increase in running wheel activity upon food restriction has been shown to be susceptible to individual differences as well as varying across sex and strain. While such differences will be discussed in more detail in later chapters, it should be noted that female rats in the ABA procedure have been shown to be more active compared to their male counterparts (Schalla & Stengel, 2019) which is thought to be consistent with the higher prevalence of AN in females compared to males. ABA, compared to other models of AN, has also been considered as the most appropriate model of self-starvation. However, while the initial reports of ABA by Routtenberg and Koznesof (1967) described lower food intake in active rats compared to inactive rats, subsequent researchers employing the ABA model have often failed to replicate this result, finding instead that active rats eat the same amount, if not more, than inactive rats. Despite the difficulty in replicating this discrepancy in food intake between active and inactive rats, contemporary ABA researchers typically argue that, while ABA animals do not necessarily eat less than control rats, ABA animals fail to consume sufficient calories to compensate for their increased energy expenditure, resulting in severe and life-threatening

weight loss. Indeed, when exposed to ABA, many rodents continue to run during food availability, thereby promoting voluntary self-starvation (Barbarich-Marsteller et al., 2013).

Biological effects of ABA. ABA has been shown to induce biological alterations relevant to AN, many of which are discussed in detail in a recent systematic review by Schalla and Stengel (2019) and are beyond the scope of this introduction. Of particular relevance is the cessation of the estrous cycle in female rats exposed to ABA (Watanabe, Hara, & Ogawa, 1992) which mimics the common presence of amenorrhea in women with AN. ABA has also been shown to result in gastrointestinal alterations such as delayed gastric emptying (Nobis et al., 2018) which is present in humans with AN and plays an important role as a maintaining factor. Similarly to 16% of individuals with AN who develop stomach ulcers (Westmoreland, Krantz, & Mehler, 2016), ABA rats have been shown to develop ulcers in the glandular portion of the stomach when weight loss exceeds 30% of their initial body weight (Doerries, Stanley, & Aravich, 1991). Additionally, ABA results in hypothermia (Paré, 1977) and alterations in the circadian sleep-wake cycle (Watanabe, Hara, & Ogawa, 1990) which resembles early morning wake, sleep, and body temperature disturbances in AN (Wakeling & Russell, 1970). ABA also mimics several endocrine abnormalities that, as in the human condition, are secondary physiologic adaptations to starvation. Such changes include decreased leptin and increased ghrelin (Boersma et al., 2016). Neurochemical and pharmacological studies, discussed in later chapters, have also shown alterations in DA and serotonin in rodents exposed to ABA (Klenotich, Ho, McMurray, Server, & Dulawa, 2015; Klenotich et al., 2012; Liu et al., 2012; Routtenberg & Kuznesof, 1967; Verhagen, Luijendijk, Korte-Bouws, Korte, & Adan, 2009). While neuroimaging studies examining brain morphology in ABA rodents are scarce, one study combining longitudinal MRI and immunohistochemical staining demonstrated a reduction in the brain volumes of ABA animals compared to controls which appeared to be driven by a 50% reduction of astrocytes in the cerebral cortex and corpus callosum. As in humans with AN, this starvation-induced brain atrophy was almost completely reversed upon refeeding (Frintrop et al., 2019).

Psychological effects of ABA. Alterations in psychological processes have also been shown to be present in ABA and are discussed in further detail in relevant chapters. Studies that have examined relevant psychological processes in ABA animals focused primarily on anxiety,

impulsivity, compulsivity, and reward processing. While these processes have generally been shown to be altered in ABA, results are mixed and inconclusive. The drawing of conclusions in this area has been largely limited by the variability in behavioural paradigms used to assess these processes, the differences in ABA protocols used, and the heterogeneity of subject demographics (e.g., sex, age, strain).

Limitations and gaps in the ABA literature

Since the first reports of ABA, researchers have come a long way in establishing ABA as the dominant and most relevant behavioural model of AN-like symptoms in rodents. Nonetheless, the ABA model itself, as well as its implementation, has been riddled with important limitations which have slowed its potential and contribution to the AN literature.

Heterogeneity of ABA protocols

While it can be concluded that ABA in rodents shares numerous similarities with AN in humans, the heterogeneity of the protocols used to induce ABA have made it difficult to compare results across studies. Indeed, several factors such as pre-exposure to the running wheel and/or feeding schedule, ambient temperature and sound, handling of animals, diet, and feeding schedule, are only examples of the many factors that have been shown to potentially affect food intake, activity, and weight loss during the development of ABA (Schalla & Stengel, 2019). The duration of ABA as well as the number of bouts of ABA varies across studies. The standard and most widely used ABA protocol has traditionally involved one ABA episode (period of restricted feeding and access to a running wheel) until animals reach survival criterion, usually within a short 5–10-day period. More recently, there have been efforts to develop ABA timelines that capture the chronicity of AN in humans by exposing animals to a period of weight reduction followed by a lighter food restriction aimed at maintaining the animals' body weight at a given number (e.g., Frintrop et al., 2018; Paulukat et al., 2016). Such protocols arguably better capture the chronic nature of AN than the time-limited standard ABA protocol. Other researchers have focused on modeling the relapsing nature of AN and have suggested re-exposing animals to a second ABA episode following a first ABA episode and subsequent recovery period (Wable, Min, Chen, & Aoki, 2015). In addition to the variability in ABA protocols used, there has been significant inconsistency in the chosen time window during which variables of interest are measured. Animals have been tested on variables of interest (e.g., DA levels, anxiety-like

behaviours) either before exposure to ABA, during acute ABA, during recovery from ABA, or during a prolonged period following ABA. These various adaptations of the ABA protocol, though necessary and informative, complicate comparisons across studies.

Subject variability

In addition to the multitude of ABA protocols employed, the significant demographic variability of the subjects used in ABA studies has also complicated the interpretation and comparison of results. The ABA protocol has been used in both rats and mice as well as in different strains. Another important problematic demographic is the use of male animals in many of the early ABA studies including those on which the ABA model was founded. Given that the large majority of individuals with AN are female, there has been a shift in the ABA literature towards increased use of female animals. Subject age also varies across ABA studies and complicates comparisons. Due to the adolescent/early adulthood onset of AN in humans, there have been efforts by ABA researchers to use adolescent rodents as subjects. Behavioural studies during rodent adolescence are challenged by the narrow time window of this developmental stage which spans approximately 14 days in rats (Spear, 2000). For this reason, many research groups opt to examine ABA in adult rodents, complicating comparisons with studies using adolescent animals.

Short survival span during ABA

When animals are exposed to ABA, they very rapidly increase their running wheel activity, fail to adjust their food intake to compensate for increased activity, and rapidly lose weight to the extent that death may ensue if they are not removed from the experiment. While this intense and rapid effect is the very strength of the ABA model, it also serves as one of the model's most limiting features. A major challenge for neurobehavioural scientists interested in elucidating the mechanisms that are in play during acute ABA is to collect the necessary data during the extremely limited time window before which an animal must be ethically removed from the experiment. This major limitation has likely deterred more than one scientist and the number of behavioural experiments using ABA are scarce as a result. Of those few studies that have taken on this logistic challenge, behavioural analyses have typically involved straightforward tasks that require limited, if any, training of the animals (e.g., elevated plus maze, EPM). More time-consuming paradigms assessing more complex constructs such as impulsivity,

compulsivity, and reward sensitivity have been conducted prior to or following recovery from ABA or avoided altogether.

Individual differences in response to ABA

A final important limitation that is of particular relevance for the present dissertation is the scarcity of research on individual differences in the development of ABA. As previously described, when rodents are exposed to severe food restriction and unlimited access to a running wheel, animals will increase their running activity and fail to adjust their food intake to compensate for the increased activity, resulting in severe weight loss. This phenomenon is the very definition of ABA. Less discussed, however, is the existence of a subgroup of rodents who, under these same conditions, either do not engage in excessive running activity or increase their food intake to compensate for their increased energy expenditure, therefore preserving their body weight. These animals that fail to develop ABA are often ignored or excluded from studies. Using these ABA-resilient animals as control groups would allow for the study of resilience and susceptibility to the development of ABA which may shed light into individual differences in AN in humans. Thankfully, over recent years and since the completion of the experiments presented in this dissertation, it has become more common practice to compare resilient and susceptible rats in ABA studies.

Rationale for current studies

The overarching goal of the present dissertation was to further the understanding of the ABA model of AN-like symptoms. More specifically, we hoped to contribute to the understanding of risk and susceptibility to AN by investigating for behavioral and neurobiological differences between rats that are most susceptible to the development of ABA and those that are most resilient.

The goal of the first set of experiments in Chapter 3 was to establish the use of the ABA model for the first time in our laboratory. We were particularly interested in optimizing the model by varying the many parameters discussed above including the use of a wheel acclimation phase, sex of the animals, and feeding duration. In this chapter, we also sought to test the feasibility of using behavioural paradigms assessing relevant psychological constructs (anxiety, depression, cognition) without interfering with ABA development.

In Chapter 4, we turned our focus towards ABA resilience and susceptibility. In this set of experiments, we were particularly interested in identifying trait differences in baseline anxiety- and depression-like behaviour, DA activity before and after ABA, and treatment response. To assess baseline anxiety- and depression-like behaviours, we tested the rats on the EPM, forced swim test (FST), and sucrose preference test (SPT) prior to exposure to ABA. In a second experiment, we examined rats' locomotor activity following varying doses of AMPH as a proxy for mesolimbic DA activity both before and after exposure to the ABA procedure. Finally, we attempted to examine for differences in treatment response between resilient and susceptible rats using olanzapine (OLZ), an atypical antipsychotic drug commonly used in the treatment of AN.

In Chapter 5, we continued our investigation of ABA resilience and susceptibility by focusing on key brain areas and relevant behaviours. More specifically, we used Fos expression as a measure of neural activity to compare activity between resilient and susceptible rats in relevant brain regions including regions of the prefrontal cortex (PFC), nucleus accumbens (NAcc), granular insular cortex, amygdala, and hypothalamus. We then examined if ABA susceptibility was associated with deficits in response inhibition, a facet of impulsivity known to be dependent on the PFC.

Finally, in Chapter 6, we assessed for differences in addiction-like behaviours between resilient and susceptible rats and, more broadly, examined the effects of a history of ABA on these behaviours. To do so, we designed the first ABA study incorporating a drug self-administration and reinstatement procedure. This procedure allowed us to test whether a history of ABA would result in a heightened motivation to work for cocaine infusions and a heightened risk for relapse and to determine how the effect of previous ABA effects these behaviours differently across resilient and susceptible rats. Together, the studies presented in this dissertation will provide insight into, not only the ABA model, but into ABA resilience and susceptibility.

CHAPTER 2: GENERAL METHODOLOGY

Subjects

A total of 24 male rats (Chapter 3) and 162 female rats (Chapter 3: $n = 60$; Chapter 4: $n = 36$; Chapter 5: $n = 36$; Chapter 6: $n = 30$) were used in various experiments. The strain of rats used in Chapter 3 was Long-Evans, while those used in all other experiments were of the Sprague Dawley strain. All rats were purchased from Charles River Laboratories (Saint-Constant, Quebec). Throughout the experiments, rats were kept on a 12:12 hr reverse light/dark cycle (housing details are described below). In standard circadian notation, zeitgeber time (ZT) 0 denotes the time when environmental lights were turned on, and ZT 12 represents the time when the lights were turned off. With the exception of the restriction phases (described below) and behavioural training phases outlined in relevant chapters, rats had *ad libitum* access to both food (Agribrand Purina Canada Inc., Woodstock, ON) and water. All animals were treated in accordance with the guidelines of the Canadian Council on Animal Care and approval for all procedures was granted by the Concordia University Animal Research Ethics Committee.

Running Wheel Cages

Running wheel cages consisted of shoebox cages (48.2 cm x 26.7 cm) with metal grid flooring. Each cage was equipped with a stainless-steel running wheel (35.0 cm in diameter) which was mounted to one side of the cage 2.8 cm above the floor. A water bottle and food hopper sat atop the cage. All running wheel activity was monitored throughout the experiment using MATLAB software (MathWorks Inc.). Each running wheel cage was individually located inside a sound-attenuating box with a louse light above the cage.

General Procedure

The experiments presented in the following chapters all followed similar general procedures (specific details are described in each chapter). Three main phases were present in all experiments: an acclimation phase, a running wheel habituation phase, and a restriction phase. Throughout all phases, rat's body weight, food intake, and water intake were monitored daily between ZT 11-12.

Acclimation Phase. Upon arrival to the animal care facility, rats were kept in an air-controlled colony room (21°C) and were pair-housed in clear shoebox cages for the first 48 hours. They were then separated into individual shoebox cages and allowed a minimum of 5 days to acclimate to the animal colony before being transferred to the running wheel cages.

Running Wheel Habituation Phase. Following the acclimation phase, rats were transferred to the laboratory where they were permanently housed in the running wheel cages for the remainder of the experiment. During this phase, rats had constant access to their running wheel and had *ad libitum* access to food and water. Rats remained under a 12:12 hr light/dark cycle, though the timing of dark onset at times shifted from the Acclimation Phase. In such cases, rats were given sufficient days to acclimate to the shift in dark onset. Running wheel activity was monitored regularly to ensure that activity was stable for a minimum of 4 days before moving to the next phase of the experiment.

Restriction Phase. During the restriction phase, access to the wheel, food, or both, was restricted, depending on the specific experiment's design. With the exception of experiments 1.3 and 1.5 (Chapter 3), food restriction lasted for 23 hours/day. The first day of food restriction began with the removal of the food at ZT 13. On subsequent days, rats had access to a pre-weighed amount of food between ZT 12-13 after which point the food was removed and weighed. Rats were sacrificed or allowed to recover once they had lost 25% of their initial body weight – days to 25% weight loss is referred to as “survival time”. In experiments with two restriction phases, rats were allowed to recover following the first restriction phase (*ad libitum* access to food) for a minimum of 7 days before undergoing a second period of food restriction as described above.

The design used during this phase varied across the different experiments but always consisted of one of the following three between-subject designs:

Two-Group Design: Sedentary vs. Active Groups. On the last day of the running wheel habituation phase, rats were assigned to either the active condition or the sedentary condition. Groups were counterbalanced based on running wheel activity and body weight. Both groups underwent food restriction, but the sedentary group's wheels were locked while the active group continued to have unlimited access to the running wheel.

Four-Group Design: Sedentary-Sated vs. Sedentary-Food Restricted vs. Active-Sated vs. Active-Food Restricted. In addition to the sedentary-food restricted and active-food restricted groups described above, two sated control groups were added to this design. In the sedentary-sated group, wheels were locked and rats continued to have *ad libitum* access to food. In the active-sated group, rats continued to have unlimited access to both the running wheel and food.

Two-Group Design: ABA-Resilient vs. ABA-Susceptible. At the start of the restriction phase, all rats in this design were food restricted while having continuous access to the running wheel. Susceptibility to the development of ABA was then determined based on one or more of the following criteria: 1) survival time, 2) wheel running activity during the running wheel habituation phase, 3) wheel running activity during the restriction phase.

Behavioural Tasks

Many behavioural tasks were used throughout this dissertation and are discussed in their respective chapters. The FST and EPM, however, were used repeatedly across several chapters and are therefore described here. All details specific to each experiment are described in the relevant chapters.

Forced Swim Test.

Apparatus. Cylinder vases made of clear glass and measuring 50.0 cm in height and 20.0 cm in diameter were used for FST. The cylinders were filled with water (25 - 28°C) up to 30.0 cm (20.0 cm from the rim). Three cylinders were placed next to each other and were separated by white Coroplast dividers. A webcam was placed in front of the cylinders and connected to a laptop in a separate room, allowing for live monitoring. All test sessions were video recorded and later scored manually.

Procedure. On the FST habituation day, rats were placed in the water-filled containers and allowed to swim for 15 min. On the following test day, rats were tested for 5 min. After the habituation and test sessions, rats were dried and returned to their running wheel cage. None of the animals drowned or struggled beyond what is expected as normal escape behaviours. Video recordings were later reviewed and immobility time (s), latency to immobility (s), and climbing (s) were scored. Immobility time represented the time during which the rat remained floating with its head just above the surface and making minimal movement to stay buoyant.

Elevated Plus Maze.

Apparatus.

The maze consisted of a wooden “plus”-shaped structure elevated 50 cm above the ground. The maze was equipped with four extending arms (11.5 x 55.0 cm) positioned at right angles. Two opposing arms had 40-cm high walls and consisted of the “closed” arms, while the other two opposing arms did not have walls and consisted of the “open” arms. The maze was kept in a dimly lit room throughout testing. A webcam was attached to the ceiling above the maze and connected to a laptop in a separate room, allowing for live monitoring. All test sessions were video recorded and later scored manually.

Procedure. Rats were transported to the testing laboratory in individual shoebox cages 1 hour prior to testing to habituate to the novel environment. At the beginning of each test, rats were placed in the center of the maze and facing an open arm. Rats were allowed to roam freely throughout the maze for 10 minutes. The maze was wiped clean with 70% ethanol between each test. Three variables were later observed and scored as follows: 1) time spent on closed arms: all paws are in closed arms or the two front paws and more than half of the body are stretched into the closed arms; 2) time spent on open arms: all paws are in open arms or the two front paws and more than half of the body are stretched into the open arms; and 3) number of closed arm entries: number of closed arm entries from open arm.

Statistical Analyses

All statistical analyses were conducted using SPSS (IBM, SPSS Statistics, version 20). Before conducting statistical analyses, all variables of interest were assessed for data entry or measurement errors, missing cases, or outliers with values greater than 3 standard deviations of the mean. Throughout the dissertation, data was analysed using a series of *t*-tests and/or analyses of variance (ANOVA)s. Effect sizes are reported using the cohen’s *d* for all *t*-tests and eta squared for all ANOVAs. ANOVAs were preceded by Mauchly’s sphericity test and a Greenhouse-Geisser correction was used when statistical significance was found. Statistically significant main effects and interactions are reported for $p \leq 0.05$. All further details specific to each experiment are described in their respective chapters.

**CHAPTER 3: ABA DEVELOPMENT AND ITS EFFECT ON MEASURES OF
PHYSIOLOGICAL AND PSYCHOLOGICAL FUNCTIONING IN LONG-EVANS MALE
AND FEMALE RATS**

ABSTRACT

Activity-based anorexia (ABA) is an animal model of anorexia nervosa (AN)-like symptoms in which rodents are food restricted while given continuous access to a running wheel resulting in hyperactivity, reduction in caloric intake, and rapid weight loss. Since the first reports of ABA by Routtenberg and Koznesof (1967), the model has been used in both male and female mice and rats of various ages and strains. The series of experiments presented in this chapter consisted of pilot projects aiming to establish the use of ABA in Long-Evans rats in our laboratory. In experiment 1.1, Long-Evans male rats were allowed to habituate to the running wheel before beginning the food restriction phase (90 min food access/day). During food restriction, ABA rats had access to the wheel while sedentary control rats had locked wheels. ABA rats showed a robust ABA response whereby they increased their running activity upon food restriction, consumed the same amount as the sedentary control rats, and reached starvation criterion within 8 days. The forced swim test (FST) was used to assess for changes in depression-like behaviours. Although the results were uninterpretable due to baseline differences, we found that it was possible to use the FST without interfering with the development of ABA. Experiments 1.2-1.4 were conducted in female rats. In experiment 1.2, rats exposed to ABA with a 60 min/day feeding window showed a robust ABA response characterised by hyperactivity and accelerated weight loss, reaching starvation criterion within 5 days. By extending the feeding window to 90 min/day in experiment 1.3, rats again showed accelerated weight loss, but hyperactivity was less present and rats did not reach starvation criterion after 9 days of ABA suggesting that the 60 min/day feeding schedule is more appropriate for producing ABA in female Long-Evans rats. In experiment 1.4, set-shifting ability, a component of cognitive flexibility, was assessed and no differences were found between rats exposed to ABA and sated control rats. Potential explanations for these results are discussed in respective experiment discussions.

CHAPTER INTRODUCTION

In their frequently cited publication from 1967, Routtenberg and Kuznesof described core symptoms of AN in a rat model, namely hyperactivity, reduced food intake, and weight loss. In these seminal experiments, Routtenberg and Kuznesof (1967) examined the effects of food restriction on running wheel activity in male albino rats. In one particular experiment, the authors examined the effects of various food restriction schedules on active and sedentary rats. A group of active rats was housed in cages equipped with a running wheel while a group of control rats were housed in standard shoebox cages. These groups were further divided into three conditions characterized by different durations of food access/day: 30 min, 45 min, or 60 min. During the experiment, all rats had continuous access to water and those in the active condition had unrestricted access to their running wheel. At the time of daily feeding, rats were restricted to a feeding area where they had *ad libitum* access to food for the predetermined amount of time. Rats were kept under these conditions for a total of 7 days or until they met the “starvation criterion” defined as eating less than 1 g during the feeding window. What emerged was the paradoxical observation that active rats ate less than the sedentary control rats and that this reduction in food intake was accompanied by increasing levels of daily wheel running. Not surprisingly, the combination of reduced food intake and increased activity propelled the rats into a negative energy balance resulting in a greater reduction in body weight in active rats compared to the sedentary control rats. The authors also concluded that a daily food access window of 60 min was ideal to obtain simultaneous self-starvation in the active rats and survival in the sedentary rats. The ability of sedentary rats to stabilize their body weight and survive pointed towards the importance of activity and accompanying negative energy balance in this animal model. Feeding windows of 30 min and 45 min were concluded to be too extreme as they resulted in starvation in both conditions (Routtenberg & Kuznesof, 1967).

While the early reports of ABA described above reported that food-restricted rats with access to a wheel consumed less than food-restricted sedentary rats, the replicability of this specific effect is uncertain. In fact, many of the earlier studies following the establishment of ABA did not have the proper control groups to determine whether this reduction in food intake in active rats, compared to sedentary rats, was replicable. Therefore, one of our first general questions as we prepared to establish the use of the ABA model in our laboratory was to examine

whether we could replicate the reduction in food intake (relative to control rats), increased running wheel activity, and accelerated weight loss in male rats that was initially reported by Routtenberg and Kuznesof (1967).

Since the first reports of ABA, a plethora of different protocols have been used, making it a challenge to compare results across studies or to establish its use in a new setting. Important parameters that have varied across studies and that have been shown to influence food intake, activity, and weight loss during ABA include pre-exposure to the feeding schedule, the presence and length of a period of habituation to the running wheel, timing of the feeding period, severity and type of food restriction employed, and survival criterion used (reviewed in Schalla & Stengel, 2019). Furthermore, although the original reports of ABA were in male albino rats, ABA has since been reported in mice, different rat strains, as well as in female rodents. These demographic variables have also been shown to affect the development of ABA. As such, another general goal of the experiments presented in this chapter was to identify the protocol and parameters that were optimal to the development of ABA in our hands.

One consistent finding across ABA studies has been the reliability in the speed at which rats lose weight when exposed to the ABA procedure, so much so that rats starve if not removed from the experiment. While the robustness of this effect is a strength of the ABA model, it also constitutes one of its greatest weaknesses as it provides a very limited time window in which other behaviours can be observed in the presence of ABA. In the original experiments by Routtenberg and Kuznesof (1967), rats were removed from the experiment when they ate less than 1 g of food/day. Using this survival criterion, the authors reported that rats survived a minimum of 7 days and up to 14 days (Routtenberg & Kuznesof, 1967). In order to avoid the confounding variable of stomach ulcers that were later shown to develop after a rat loses 30% of its initial body weight (Doerries et al., 1991), the majority of researchers have since used 25% weight loss as the survival criterion. Using this more conservative criterion, survival duration under ABA has ranged from 3 to 10 days (Schalla & Stengel, 2019). Of course, survival is influenced by many factors such as duration of feeding window (traditionally 1-1.5 h), timing of feeding, handling, sex, strain, ambient temperature, pre-exposure to feeding schedule, etc. (Schalla & Stengel, 2019). Thus, an important goal of the experiments presented in this chapter

was to identify the parameters that would result in a robust ABA effect while also maximizing survival duration.

The limited time window in which rodents can survive under ABA conditions undoubtedly complicates any attempt to use additional behavioural paradigms to assess AN-related symptoms during ABA. Depression and impaired cognitive functioning, for instance, are both common features of AN but have rarely been investigated in the ABA model, presumably because of the logistic challenges of carrying out such behavioural assessments in such limited time. A goal of the experiments in this chapter was therefore to begin testing the feasibility of using behavioural assessments in conjunction with ABA and, more specifically, to determine whether depression-like symptoms and cognitive impairment are present in ABA.

In summary, the series of experiments presented in this chapter consisted of several pilot projects aiming to establish the use of the ABA procedure in our laboratory. The first two experiments were designed to examine the effects of pre-exposure to the feeding schedule and pre-exposure to the running wheel on ABA development in male rats. Due to the preliminary nature of these experiments, they have been included as supplementary experiments (see Appendix 1 and 2). The experiments presented here investigated additional parameters (e.g., feeding duration, sex) in an effort to produce a robust and useful ABA effect. We also aimed to begin testing the feasibility of using other behavioural paradigms during ABA without disrupting its development.

EXPERIMENT 1.1. THE EFFECT OF A 22.5-HOUR FOOD RESTRICTION IN ACTIVE VERSUS SEDENTARY LONG-EVANS MALE RATS

In a preliminary study (presented in Appendix 2), male rats were pre-exposed to the running wheel before starting the food restriction. Under these sated conditions, we observed a temporary reduction in food intake accompanied by weight loss, not unlike the wheel-induced feeding suppression previously reported by other researchers (e.g., Afonso & Eikelboom, 2003; Goodrick, Ingram, Reynolds, Freeman, & Cider, 1983; Looy & Eikelboom, 1989). This wheel-induced feeding suppression has been suggested to capture voluntary feeding suppression that better mimics food restriction in AN rather than the forced food restriction in the ABA model (Afonso & Eikelboom, 2003). While the wheel-induced feeding suppression we observed had a large effect size, it did not reach statistical significance. In the present experiment, we sought to increase statistical power by increasing our sample size to examine if the wheel-induced feeding suppression would be replicated.

The onset of the ABA phase (simultaneous wheel access and food restriction) in our preliminary study (presented in Appendix 2) resulted in a significant increase in running activity accompanied by rapid weight loss, both central features of the ABA effect. While the rats were not consuming enough calories to compensate for their increased energy expenditure, as evidenced by rapid weight loss, we could not determine whether an anorectic effect was present given the absence of a control group. Here, we included a sedentary control group that would undergo the same food restriction during the ABA phase as the active group, but that would not have access to the running wheel. This allowed for a direct comparison of food intake during the ABA phase.

Furthermore, a feeding window of 60 min was used in our preliminary study (presented in Appendix 2) and rats were found to reach starvation criterion (75% of initial body weight) within 7 days. This survival duration is consistent with those reported by other researchers who have also used this starvation criterion and a 60 min feeding window in male rats (e.g., Aoyama, 2012; Pardo et al., 2010; Ratnovsky & Neuman, 2011). One goal of the present experiment was to test whether survival duration could be prolonged by extending the feeding window to 90 min while not compromising ABA development.

The final goal of the present experiment was to begin exploring the effect of ABA on depression-like behaviours. Given the limited time that rats can ethically be kept under ABA conditions, conducting time-consuming behavioural paradigms during ABA poses a challenge. To the best of our knowledge, depression-like behaviours have not been examined in the context of ABA. The FST was developed as a model for predicting the clinical efficacy of antidepressants (Porsolt, Bertin, & Jalfe, 1977) and is often used as a measure of depression-like behaviour in rodents. In this test, immobility becomes present as the session progresses and can be interpreted as behavioural despair. Importantly, rats do not need to be trained for FST and the procedure can be carried out across two days thus making it possible to use with the time-limited ABA procedure. Given that depression is a common comorbidity in AN, we expected to see increased depression-like behaviours during ABA. More specifically, we expected to see longer immobility time and shorter latency to immobility in active rats compared to sedentary rats during the ABA phase only.

In summary, we had three sets of hypotheses for the present experiment. During the running wheel-sated phase, we expected to observe a temporary wheel-induced feeding suppression accompanied by weight loss. We also expected that running wheel activity would initially increase daily before reaching a stable level. During the ABA phase, we hypothesized that active rats would increase their running wheel activity. We also hypothesized that active rats would eat equal amounts to or less than sedentary control rats and that they would show accelerated weight loss. We hypothesized that rats would require more than 7 days to reach the starvation criterion. Finally, with regards to the FST, we hypothesized that active rats would show more depression-like behaviour compared to the sedentary control rats during the ABA phase.

Method

Subjects

Male Long-Evans rats ($n = 12$; 325-350 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec) and housed in a colony room on a 12:12 hr reverse light/dark cycle. Upon arrival, rats were initially pair-housed in plastic shoebox cages and were then separated into individual shoebox cages (day 1). On day 4, rats were transferred to the laboratory where they were permanently housed in running wheel cages inside sound-attenuating

boxes until the end of the experiment. At the onset of the food restriction phase (described in the procedure section below), rats were assigned to one of the following two conditions: sedentary condition ($n = 6$) or active condition ($n = 6$). Running wheel activity, food intake, and body weight were matched across both conditions. With the exception of the food restriction phase, rats had *ad libitum* access to both food and water throughout the experiment and body weight (g), food intake (g), and water intake (g) were monitored daily at ZT 11-12. Weight loss was used as the starvation criterion and rats were removed from the experiment when they had lost 25% or more of their initial body weight.

Apparatus

Running Wheel Cages. See “General Methodology” section.

Forced Swim Task. See “General Methodology” section.

Procedure

The detailed timeline is depicted in Figure 3.1.

Acclimation phase. Rats were individually housed in plastic shoebox cages for 4 days in the Animal Care Facility, allowing them time to acclimate to the new environment. This period also allowed for baseline daily measures of body weight, food intake, and water intake.

Wheel habituation phase. Following the 4 acclimation days, rats were transferred to the individual running wheel cages where they were permanently housed for the remainder of the experiment. During this phase, all rats had continuous access to the running wheel and *ad libitum* food and water. The wheel habituation phase was continued for a total of 24 days at which point running wheel activity had stabilized for 4 consecutive days.

Food restriction phase. At the onset of the food restriction phase, a metal rod was used to lock the running wheels of rats in the sedentary condition while wheels of rats in the active condition remained unlocked. Food was removed for all rats at ZT 13.5. On the following days, rats had access to food for 1.5 hr/day between ZT 12-13.5. The experiment was terminated after 10 days of the food restriction phase.

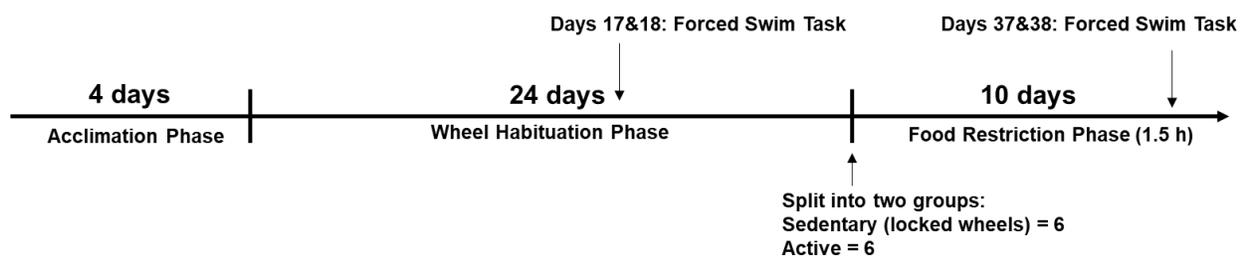


Figure 3.1. Timeline of experiment 1.1.

Forced Swim Task. Rats underwent the FST procedure on two occasions. They were first tested during the wheel habituation phase (i.e., pre-food restriction FST) and later tested during the food restriction phase (i.e., food restriction FST). The task was administered during the active phase (ZT 15-18) under regular light conditions. See “General Methodology” for more details.

Statistical Analysis

Running wheel activity. A two-way mixed ANOVA using *days* as the within-subjects factor and *wheel condition* as the between-subject factor was used to characterize wheel activity during the wheel habituation phase. A two-way mixed ANOVA examining the 4 final days of the habituation phase (*days*) as the within-subjects factor and *wheel condition* as the between-subjects factor was then used to determine whether rats’ running wheel activity had stabilized. A paired *t*-test was used to compare the average daily running wheel activity during the last 4 days of the wheel habituation phase and the first 4 days of the food restriction phase in the active group (the sedentary group was not included in this analysis as they did not have access to the wheel during the food restriction phase).

Food intake. A paired *t*-test was used to compare food intake averaged across the 4 days of acclimation to that of the first 4 days of the wheel habituation phase. A 2 x 24 mixed ANOVA was then used with *wheel condition* as the between-subject factor and *days* as the within subject factor to characterize food intake across the 24-wheel habituation days. To verify that food intake had stabilized by the end of the wheel habituation phase, a 2 x 4 mixed ANOVA was used with *wheel condition* as the between-subject factor and *days* (4 last days of the wheel habituation phase). To assess the general impact of food restriction on food intake, a paired *t*-test was used comparing food intake averaged across the last 4 days of wheel habituation phase to that of the first 4 days of the food restriction phase. In order to compare food intake during the food restriction phase between active and sedentary rats, a 2 x 10 mixed ANOVA was used with *wheel condition* as the between-subject factor and *days* (the 10 days of the food restricted phase) as the within-subject factor.

Body weight. A paired *t*-test was used to compare body weight on the last day of the acclimation phase to that of the first day of the wheel habituation phase. A 2 x 24 mixed

ANOVA was then used with *wheel condition* as the between-subject factor and *days* as the within subject factor to characterize body weight across the 24-wheel habituation days. A 2 x 2 mixed ANOVA with *wheel condition* as the between-subject factor and *phase* (body weight averaged across the last 4 days of the wheel habituation vs. body weight averaged across the first 4 days of the food restriction phase) was used to examine the effect of food restriction on body weight in active and sedentary rats. Finally, in order to compare body weight during the food restriction phase between active and sedentary rats, a 2 x 10 mixed ANOVA was used with *wheel condition* as the between-subject factor and *days* (the 10 days of the food restricted phase) as the within-subject factor.

Forced Swim Task. Time spent immobile, time spent climbing, and latency to immobility, in seconds, were noted for rats during the FST sessions. Three 2 x 2 mixed ANOVAs were used with each of the dependent variables using *wheel condition* as the between-subject factor and *phase* as the within-subject factor.

Results

Running wheel activity

Running wheel activity increased in a statistically significant way across the wheel habituation phase (*days*: $F(23, 230) = 21.50, p < .001, \eta_p^2 = .68$; Figure 3.2A). As wheel activity during wheel habituation was matched, there was no statistically significant difference in running wheel activity during the wheel habituation phase between active and sedentary rats and no *days x wheel condition* interaction (*wheel condition*: $F(1, 10) = 0.01, p = .973, \eta_p^2 < .01$; *days x wheel condition*: $F(23, 230) = 1.23, p = .221, \eta_p^2 = .11$). Running wheel activity remained stable across the last 4 days of the wheel habituation phase (*days*: $F(3, 30) = 1.86, p = .158, \eta_p^2 = .16$; *wheel condition*: $F(1, 10) = 0.06, p = .809, \eta_p^2 = .01$; *days x wheel condition*: $F(3, 30) = 0.53, p = .066, \eta_p^2 = .05$).

There was a statistically significant increase in the average of daily running wheel activity of rats in the active condition during the last 4 days of the wheel habituation phase compared to the first 4 days of the food restriction phase ($t(5) = -2.93, p = .016, d = 1.19$; Figure 3.2B).

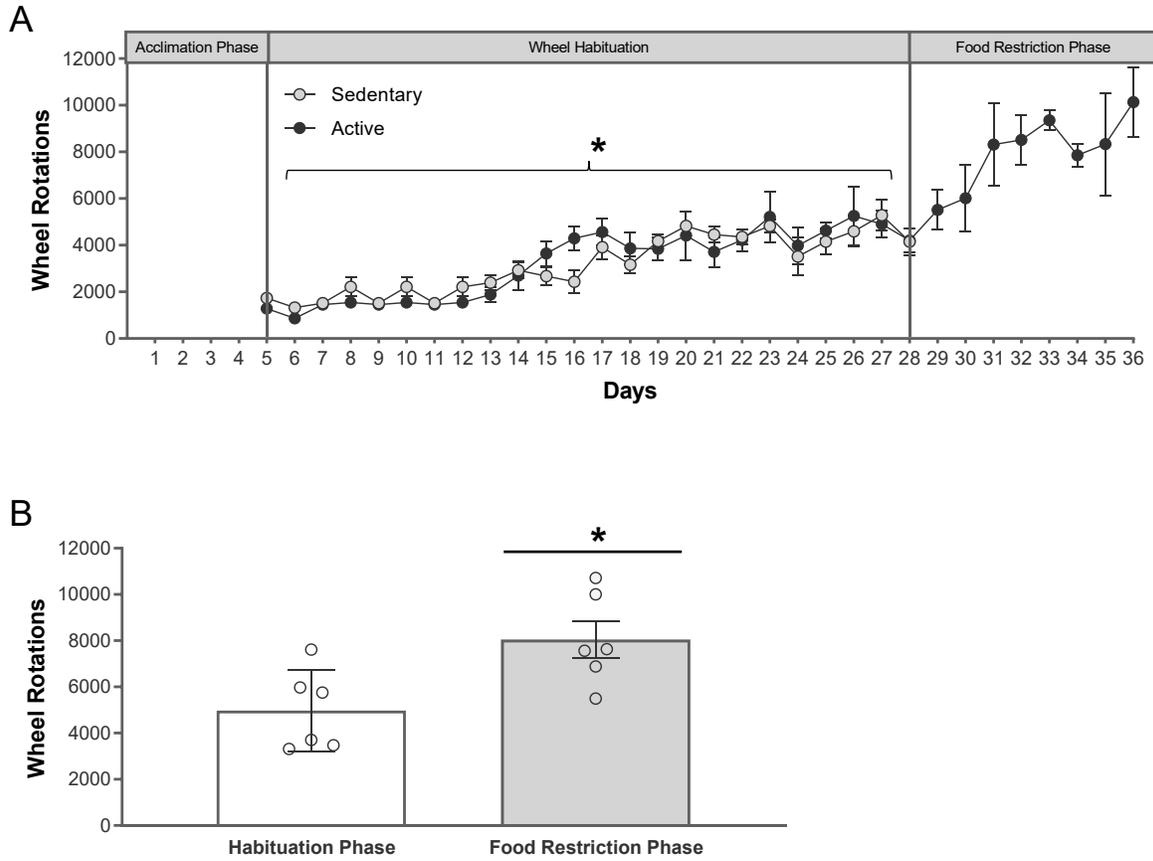


Figure 3.2. Effect of feeding schedule on running wheel activity. (A) Running wheel activity across experimental days; $*p < .001$, main effect of *days*. (B) Average running wheel activity across the last 4 days of the wheel habituation phase compared to the first 4 days of the food restriction phase, $*p = .016$.

Food intake

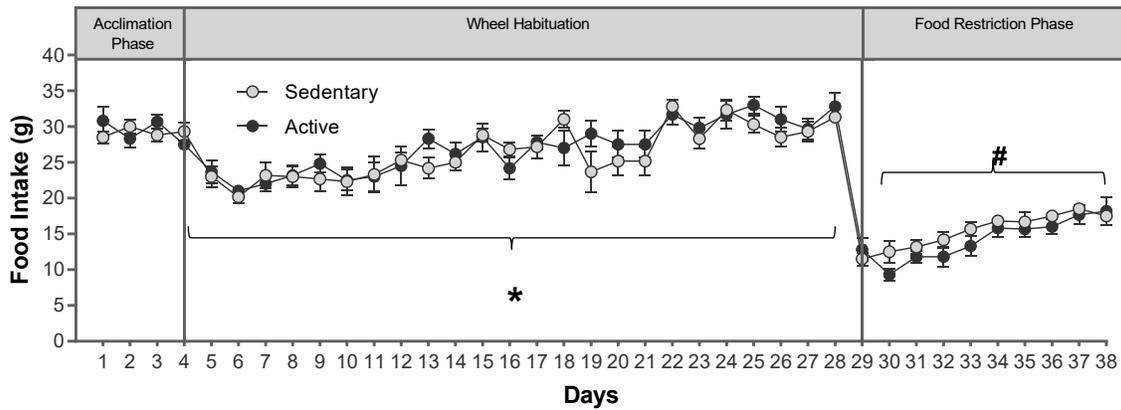
Figure 3.3A depicts food intake across experimental days. The introduction of a running wheel under *ad libitum* feeding conditions resulted in a statistically significant decrease in food intake compared to the average food intake during the 4 days where rats were housed in the animal care facility without a wheel ($t(11) = 9.15, p < .001, d = 2.64$; Figure 3.3B). This wheel-induced decrease in food intake lasted approximately 17 days with rats eating an average of 26.33 g ($SEM = 0.85$) on the 17th day of habituation compared to 28.42 g ($SEM = 0.72$) on the day before the wheel was introduced. Food intake showed a gradual and statistically significant increase across the 24 days of habituation ($days: F(23,230) = 11.76, p < .001, \eta_p^2 = .54$, Figure 3.3A). As the wheel conditions had been matched based on food intake, there was unsurprisingly no statistically significant main effect of *wheel condition* ($F(1, 10) = 0.36, p = .565, \eta_p^2 = .03$) or *wheel condition x days* interaction ($F(23, 230) = 1.06, p = .396, \eta_p^2 = .10$). By the last 4 days of the habituation phase, food intake had stabilized ($days: F(3, 30) = 2.49, p = .079, \eta_p^2 = .20$) and there was no statistically significant main effect of *wheel condition* ($F(1, 10) = 1.67, p = .226, \eta_p^2 = .14$) or *wheel condition x days* interaction ($F(3, 30) = 0.42, p = .742, \eta_p^2 = .04$).

Not surprisingly, daily food intake (across both conditions) during the first 4 days of the food restriction phase was significantly lower than food intake during the last 4 days of the wheel habituation phase ($t(11) = 18.60, p < .001, d = 5.37$; Figure 3.3C). Food intake gradually increased across food restriction days ($days: F(9, 90) = 16.70, p < .001, \eta_p^2 = .63$). There were, however, no statistically significant differences between active and sedentary rats (*wheel condition*: $F(1, 10) = 1.03, p = .334, \eta_p^2 = .09$) and no significant *wheel condition x days* interaction ($F(9, 90) = 1.19, p = .314, \eta_p^2 = .11$).

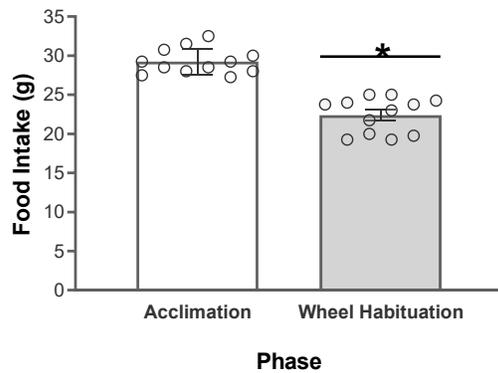
Body weight

As can be seen in Figure 3.4A, the introduction of a running wheel under *ad libitum* feeding conditions resulted in a statistically significant decrease in body weight on the first day of the wheel habituation phase compared to the last day of the acclimation phase ($t(11) = 4.88, p < .001, d = 1.41$). Body weight gradually increased in a statistically significant way across the 24 days of the wheel habituation phase ($days: F(23,230) = 37.93, p < .001, \eta_p^2 = .79$; 3.4B). As the

A



B



C

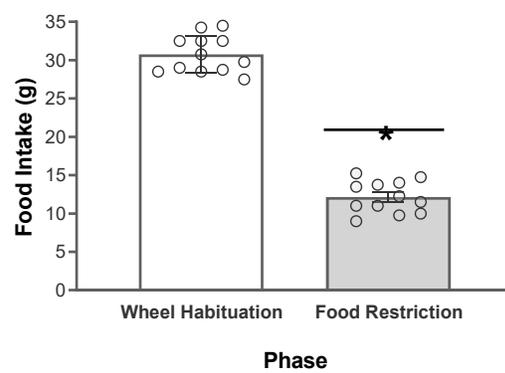
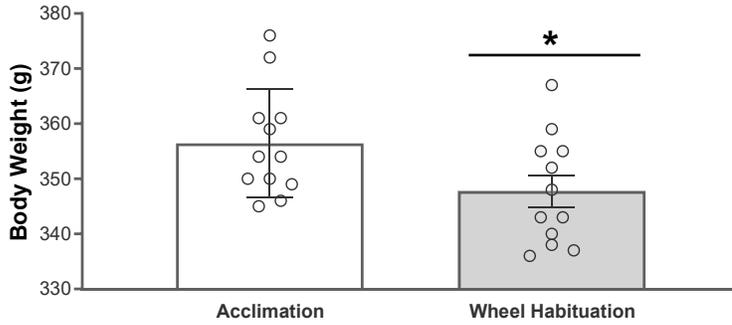


Figure 3.3. The effect of food restriction on daily food intake in sedentary and active rats. (A) Daily food intake across experimental days; $*p < .001$, main effect of *days*; $\#p < .001$, main effect of *days*. (B) Average daily food intake across the 4 days of the acclimation phase compared to the first 4 days of wheel habituation phase, $*p < .001$. (C) Average daily food intake across the last 4 days of the wheel habituation phase compared to the first 4 days of the food restriction phase, $*p < .001$.

A



B

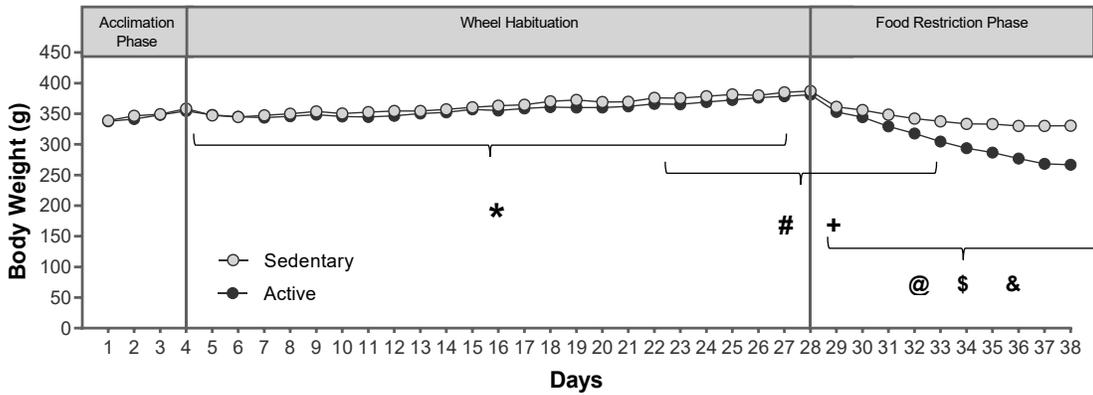


Figure 3.4. The effect of food restriction running wheel activity on daily body weight in sedentary and active rats. (A) Body weight on the last day of the acclimation phase compared to the first day of the wheel habituation phase, $*p < .001$. (B) Daily body weight across experimental days; $*p < .001$, main effect of days; $\#p < .001$, main effect of phase; $+p = .014$, phase \times wheel condition trending interaction; $@p < .001$, main effect of days; $\$p < .001$, main effect of wheel condition; $\&p < .001$, wheel condition \times phase interaction.

wheel condition had been matched based on weight, there was unsurprisingly no statistically significant main effect of *wheel condition* ($F(1, 10) = 1.24, p = .291, \eta_p^2 = .11$) or *wheel condition x days* interaction ($F(23, 230) = 0.64, p = .900, \eta_p^2 = .06$).

The beginning of the food restriction phase resulted in a significant decrease in body weight (Figure 3.4B). While the main effect of *wheel condition* was not statistically significant, there was a statistically significant *phase x condition* interaction indicating that the weight loss was accelerated in rats in the active condition compared to those in the sedentary condition who showed a slower weight loss (*phase*: $F(1, 10) = 497.47, p < .001, \eta_p^2 = 0.98$; *wheel condition*: $F(1, 10) = 2.17, p = .171, \eta_p^2 = 0.18$; *phase x wheel condition*: $F(1, 10) = 8.73, p = .014, \eta_p^2 = 0.47$).

Across the 10 days of the food restriction phase, there was a gradual and statistically significant decrease in body weight (*days*: $F(9, 90) = 113.74, p < .001, \eta_p^2 = .92$). Active rats lost significantly more weight than sedentary rats (*wheel condition*: $F(1, 10) = 29.92, p < .001, \eta_p^2 = .75$). Importantly, there was a statistically significant *wheel condition x days* interaction ($F(9, 90) = 26.03, p < .001, \eta_p^2 = .72$) suggesting that the weight loss occurred in rats in the active condition while rats in the sedentary condition managed to maintain their body weight following their initial loss on the first day of the food restriction phase..

Forced Swim Task

When examining immobility time (Figure 3.5A), rats in the active condition spent significantly more time immobile compared to rats in the sedentary condition (*wheel condition*: $F(1, 10) = 6.17, p = .032, \eta_p^2 = 0.38$). There was no statistically significant main effect of *phase* ($F(1, 10) = 0.27, p = .613, \eta_p^2 = 0.27$). There was, however, a trending *phase x wheel condition* interaction indicating that the difference between sedentary and active rats was present during the pre-food restriction test but not during food restriction (*phase x wheel condition*: $F(1, 10) = 3.93, p = .076, \eta_p^2 = 0.28$). Rats in the active condition spent less time climbing compared to rats in the sedentary condition (*wheel condition*: $F(1, 10) = 7.01, p = .024, \eta_p^2 = 0.41$, Figure 3.5B). Consistent with immobility time, there was a trending *phase x wheel condition* interaction indicating that the difference in climbing between sedentary and active rats was present during the pre-food restriction test but not during food restriction (*phase x wheel condition*: $F(1, 10) =$

4.46, $p = .061$, $\eta_p^2 = 0.31$). There was no statistically significant main effect of *phase* ($F(1, 10) = 0.39$, $p = .546$, $\eta_p^2 = 0.04$). When examining latency to immobility (Figure 3.5C), rats in the active condition showed a longer latency to immobility compared to sedentary rats, though this did not reach statistical significance (*wheel condition*: $F(1, 10) = 4.32$, $p = .064$, $\eta_p^2 = 0.30$). There was no statistically significant main effect of *phase* or *phase x wheel condition* interaction (*phase*: $F(1, 10) = 0.32$, $p = .581$, $\eta_p^2 = 0.03$; *phase x condition*: $F(1, 10) = 1.13$, $p = .314$, $\eta_p^2 = 0.10$).

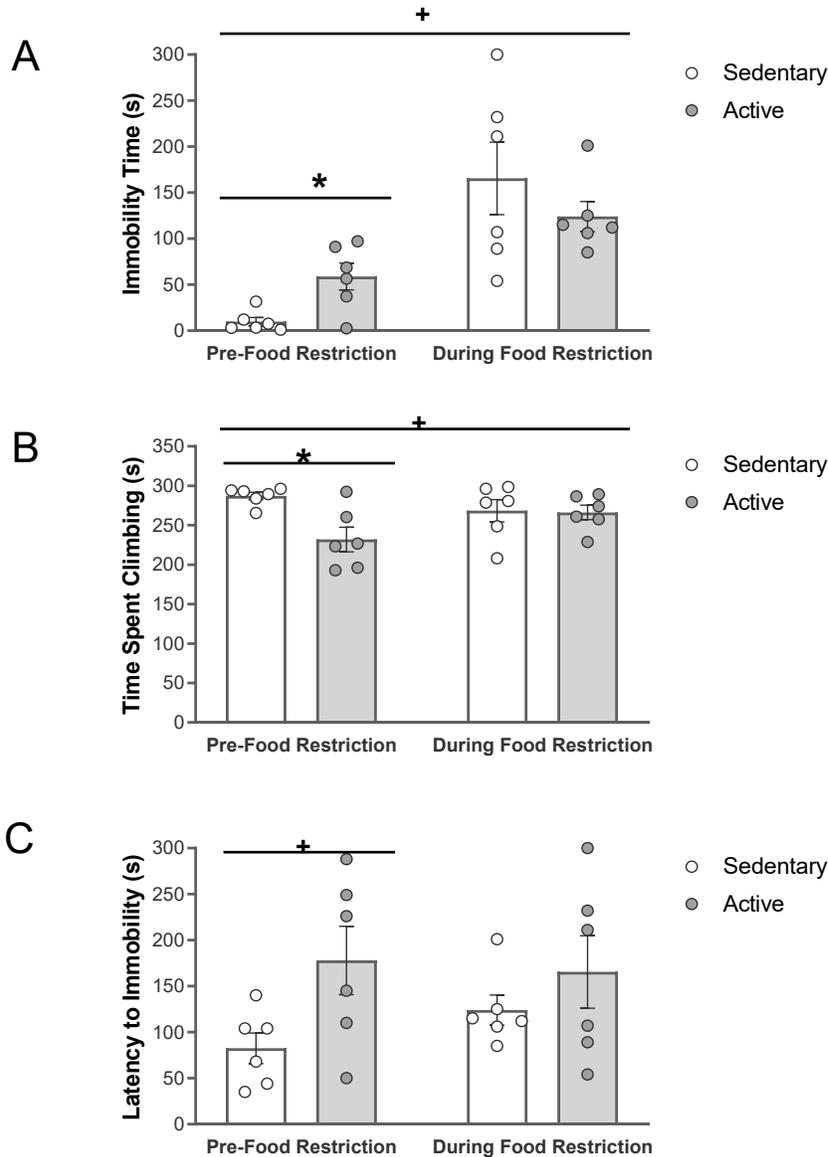


Figure 3.5. The effect of food restriction and wheel access on the FST in sedentary ($n = 6$) and active ($n = 6$) rats pre-food restriction and during food restriction. (A) Mean immobility time in seconds; $*p = .032$, main effect of *wheel condition*; $+p = .076$, trending *phase x wheel condition* interaction. (B) Mean time spent climbing in seconds; $*p = .024$, main effect of *wheel condition*; $+p = .061$, trending *phase x wheel condition* interaction. (C) Mean latency to immobility in seconds; $+p = .064$; trending effect of *wheel condition*.

Discussion

The overarching goal of the present experiment was to build on our two preliminary studies (presented in Appendix 1 and 2) by adding a sedentary control group that would not have access to the running wheel during the food restriction. This comparison enabled us to examine the effect of running wheel activity on food intake during food restriction. We also extended the feeding window to 90 min/day to examine whether this would increase survival duration during the ABA phase. Finally, we examined the effect of ABA on depression-like behaviours using the FST. Various sets of hypotheses were outlined for the different experimental phases and are discussed below.

Wheel habituation phase

As hypothesized, we observed a gradual increase in running wheel activity across the wheel habituation phase with activity reaching a stable level by the end of this 24-day phase. Compared to our preliminary study (presented in Appendix 2) which counted only 11 days for this phase, we extended the phase to 24 days to accommodate for the first FST session and to allow for recovery before beginning the food restriction phase. By the end of this wheel habituation phase, rats' activity had stabilized at approximately 4648 wheel rotations a day. This activity level was higher than that of the rats in our preliminary study (presented in Appendix 2) that reached approximately 3877 wheel rotations/day by the end of the 11-day wheel habituation phase. It was also higher than the levels typically reported in other rat strains (e.g., Afonso & Eikelboom, 2003; Ratnovsky & Neuman, 2011).

It was also hypothesized that the introduction of the running wheel under sated conditions would result in a temporary feeding suppression accompanied by weight loss. This hypothesis was supported by our observation of a wheel-induced feeding suppression that lasted 17 days. This temporary wheel-induced feeding suppression lasted longer than that reported in male Sprague Dawley rats that lasted from 3 days (Ratnovsky & Neuman, 2011) to 8 days (Afonso & Eikelboom, 2003), suggesting that this effect is more robust in Long-Evans male rats. Afonso and Eikelboom (2003) suggested that severe food restriction, as used in ABA procedures, may not be necessary to model AN as initial wheel access can induce a pronounced and voluntary feeding suppression, regardless of the feeding regime. It should be noted that, while we did observe a wheel-induced feeding suppression and initial weight loss, body weight gradually

increased during the 17 days of suppressed eating. It therefore appears that rats' caloric intake, albeit suppressed, was sufficient to compensate for their increased energy expenditure when food was provided *ad libitum*. Thus, while wheel-induced feeding suppression is an interesting phenomenon, it does not appropriately capture the severe caloric restriction and accompanying weight loss observed in AN and modeled by ABA. Instead, wheel-induced feeding suppression under sated conditions may capture the mild reduction in caloric intake reported in humans undergoing non-excessive levels of physical activity. For instance, Shaw, Shaw, and Brown (2008) found that concurrent aerobic and weight training promoted a favourable improvement in self-reported dietary intake in a sample of men.

Food restriction Phase

It was hypothesized that food restriction would result in a significant increase in running wheel activity. Indeed, we found that the beginning of the food restriction phase resulted in hyperactivity, with wheel activity peaking at approximately 10 132 wheel rotations a day by the 8th and final day of the food restriction phase. During this same time, we found that active rats consumed the same amount as the sedentary control rats. This finding is inconsistent with the early finding that active rats ate less than sedentary rats when given access to food for 30 min, 45 min, or 60 min/day (Routtenberg, 1968). Nonetheless, it is noteworthy that active rats in the present experiment did not eat more than the sedentary control rats and thus were not compensating for their increased energy expenditure. This, unsurprisingly, resulted in accelerated weight loss during the food restriction phase compared to the sedentary control rats. We had also hypothesized that the active rats would require more than 7 days to reach starvation criterion during the food restriction phase as the feeding window was increased to 90 min (compared to 60 min in our preliminary study presented in Appendix 2). We found that rats reached the starvation criterion of 25% weight loss by the 8th day of ABA, suggesting that the food restriction effect was robust despite the prolonged feeding window.

Forced swim task

It was hypothesized that ABA would result in more depression-like behaviours in the FST. More specifically, we did not expect to see any differences between conditions in immobility time and latency to immobility during pre-ABA tests because there were no differences between the two conditions at that time (i.e., all rats had unlimited access to the

wheel and food). We, however, hypothesized that active rats, compared to the sedentary control rats, would show more immobility time and shorter latency to immobility during the food restriction phase. Unfortunately, this hypothesis could not be tested because of pre-existing differences between conditions. Rats that were later allocated to the active condition were found to, overall, spend more time immobile, compared to those that were later allocated to the sedentary condition. It would therefore be misguided to interpret the FST results from the present experiment. Given the large variability observed in our FST results, it would be beneficial to increase sample size when possible. Furthermore, in future experiments, it will be crucial to match experimental conditions based on baseline FST results in order to properly investigate the effects of ABA on depression-like behaviours.

Conclusions

Overall, the results from the present experiment lend support for the use of the ABA procedure in Long-Evans male rats. We found that the introduction of the running wheel alone, under sated conditions, results in a temporary wheel-induced feeding suppression whereby rats run increasingly more and consume less calories than pre-wheel levels. We found that this wheel-induced feeding suppression was temporary and, in Long-Evans male rats, did not result in chronic weight loss suggesting that rats were eating enough to compensate for increased energy expenditure. Under ABA conditions, however, we found that Long-Evans male rats showed hyperactivity accompanied by rapid weight loss. The ABA effect was so robust that, even with a longer feeding window of 90 min, rats could not survive under these conditions for more than 8 days. Compared to our preliminary study (presented in Appendix 2), the present design included a sedentary control condition that allowed us to examine the effect of wheel activity on food intake during food restriction. While we did not replicate previous findings suggesting that active rats ate less than sedentary rats (Routtenberg, 1968), we found that active rats ate the same amount despite their hyperactivity, which drove their rapid weight loss. A limitation of the present experiment was that baseline FST behaviours were not matched when the groups were created, rendering the FST results uninterpretable. Nonetheless, we found that it was possible to run an FST procedure without interfering with the development of ABA.

EXPERIMENT 1.2. ACTIVITY-BASED ANOREXIA IN FEMALE LONG-EVANS RATS AND ITS EFFECTS ON DEPRESSION-LIKE BEHAVIOURS AND PLASMA LEPTIN LEVELS

In the previous experiment, we found that the ABA model can be used in male Long-Evans rats to mimic AN-like symptoms. Given that AN is far more prevalent in women, the main goal of the present experiment was to investigate whether ABA would also develop in female Long-Evans rats and whether it would be accompanied by depression-like behaviours and hormonal imbalances.

While some researchers have reported that female rodents are more susceptible to the development of ABA (Paré, 1977), results from ABA studies in female rodents have been mixed. Studies have indeed shown that there are sex differences in the development of ABA, though it is unclear what these differences suggest in terms of ABA susceptibility. Females exposed to ABA have been shown to have higher levels of running wheel activity than males and this hyperactivity is often reported to support the claim that the development of ABA is more robust in females. However, Boakes, Mills, and Single (1999) found that, while female rats did run more than males overall, weight loss resulted in increased running wheel activity in males only. Males and females undergoing ABA also appear to differ in food consumption whereby females reportedly eat more than males (Doerries et al., 1991). Indeed, the decreased food intake in active male rats compared to sedentary rats reported in the original ABA studies has not been replicated in female rats. Instead, when female rats have been exposed to ABA, they consume either equal amounts or more than sedentary female rats, presumably in an effort to compensate for their increased energy expenditure (Schalla & Stengel, 2019). Regardless, this effort is insufficient in preventing a negative energy balance which eventually results in severe weight loss, albeit at a slower rate than male rats. Thus, while females have been reported to show more hyperactivity than males, ABA appears to develop more effectively and rapidly in male rats. Given our interest in the ABA procedure as a model of symptoms of AN, a disorder that is most prevalent in females, we sought to further examine the manifestation of ABA in female rats.

Different strains of rats have been used in female ABA experiments and have included Wistar rats and Long-Evans rats, though the majority of studies have used Sprague Dawley rats. Studies have also varied with regards to the duration of the feeding window used, generally

ranging from 60 min to 120 min. Given this variability in protocols, it should be of no surprise that the reported number of days required to reach starvation criterion has also varied greatly, ranging from as little as 3 days to 21 days. As such, we aimed to characterize ABA development specifically in adult Long-Evans female rats.

We also sought to examine whether ABA would result in depression-like behaviours in females. As in experiment 1.1, the FST was conducted before food restriction and again during the food restriction phase. Given the large variability observed in the FST results in experiment 1.1, we hoped that the greater sample size used in the present experiment would increase our statistical power. We also sought to address an important limitation of the previous experiment by taking into consideration FST behaviours during the pre-ABA phase to match rats in the active and sedentary conditions. We expected that food restriction would result in more depression-like behaviours compared to the pre-ABA phase in active rats but not in sedentary control rats.

An additional goal of the present experiment was to examine the effect of ABA on plasma leptin levels. Leptin is a hormone that plays a central role in appetite and regulation of energy balance. It is mainly synthesized in adipocytes from where it is secreted and is thus highly correlated with body mass index. Not surprisingly, during the acute stage of AN when patients are at their lowest weight, leptin levels are below the reference range of age-matched and younger BMI-matched controls (Hebebrand et al., 1997, 1995). Interestingly, low leptin levels have been associated with increased physical activity in AN (Hillebrand, Van Elburg, Kas, Van Engeland, & Adan, 2005). In one study examining the correlation between self-reported motor restlessness and plasma leptin, patients reported the highest levels of motor restlessness when leptin levels and body weight were at their lowest (Exner et al., 2000). Given the hypoleptinemia observed in AN and its link with hyperactivity, researchers have examined the effects of leptin treatment on the development of ABA in rodents. Chronic leptin treatment via osmotic minipumps, central injections in the lateral ventricles, or local injections into the VTA have all been shown to suppress hyperactivity in ABA (Exner et al., 2000; Hillebrand, Koeners, De Rijke, Kas, & Adan, 2005; Verhagen, Luijendijk, & Adan, 2011). We were therefore interested in examining whether ABA development would be associated with decreases in plasma leptin in Long-Evans females. Plasma was collected on the final day of the ABA phase 1

hour before feeding (pre-prandial) and 10 min after feeding (post-prandial). We expected that leptin levels would be highest after feeding and that active rats would have overall lower plasma leptin compared to the sedentary control rats.

In summary, the present experiment was the first in this chapter to examine ABA development in female rats. During the wheel habituation phase, we expected that wheel introduction would result in a temporary wheel-induced feeding suppression, as was observed in our previous experiments in Long-Evans male rats. We expected to see an increase in running wheel activity in the active rats at the onset of the food restriction phase. During the food restriction phase, we expected that the active rats would eat the same amount as sedentary rats, thus failing to compensate for their increased energy expenditure resulting in accelerated weight loss. With regards to FST, we expected that active rats, but not sedentary rats, would have higher immobility time and shorter latency to immobility during the food restriction phase compared to the wheel habituation phase. Finally, with regards to plasma leptin, we expected that leptin levels would be higher after feeding and that active rats would have overall lower plasma leptin compared to the sedentary control rats.

Method

Subjects

Female Long-Evans rats ($n = 24$; 226-250 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec) and housed in a colony room on a 12:12 hr reverse light/dark cycle. Upon arrival, rats were initially pair-housed in plastic shoebox cages and left alone. On the following day (experimental day 1), rats were separated into individual shoebox cages allowing for baseline measures of daily food intake, water intake, and body weight. On day 3, rats were transferred to the laboratory where they were permanently housed in individual running wheel cages inside sound-attenuating boxes until the end of the experiment. At the onset of the food restriction phase, rats were assigned to one of the following two wheel conditions: sedentary condition ($n = 12$) or active condition ($n = 12$). Running wheel activity, food intake, body weight, and performance on the FST were matched across both conditions. With the exception of the food restriction phase, rats had *ad libitum* access to both food and water throughout the experiment and body weight (g), food intake (g), and water intake (g) were

monitored daily at ZT 11-12. Rats were removed from the experiment when they lost $\geq 25\%$ of their body weight on the day before food restriction.

Apparatus

Running Wheel Cages. See “General Methodology” section.

Forced Swim Task. See “General Methodology” section.

Plasma leptin concentration. Plasma leptin was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Millipore, MA, USA). The reported detection sensitivity for the ELISA kit was 0.04 ng/ml.

Procedure

The detailed timeline is depicted in Figure 3.6.

Acclimation phase. Rats were individually housed in plastic shoebox cages for 2 days in the Animal Care Facility before being transported to the wheel cages. This period allowed for baseline daily measures of body weight, food intake, and water intake.

Wheel habituation phase. Following the 2 acclimation days, rats were transferred to the individual running wheel cages where they were permanently housed for the remainder of the experiment. During this phase, all rats had continued access to the running wheel and *ad libitum* food and water. This phase lasted 14 days (days 3-16).

Food restriction phase. At the onset of the food restriction phase, a metal rod was used to lock the running wheels of rats in the sedentary condition while wheels of rats in the active condition remained unlocked. Food was removed for all rats at ZT 13. On the following days, rats had access to food for 1 hr/day between ZT 12-13. The experiment was terminated after 5 days of the food restriction phase.

Forced Swim Task. Rats underwent the FST procedure on two occasions: during the habituation phase (days 10-11) and during the food restriction phase (days 19-20). See “General Methodology” section for details of the FST procedure.

Plasma leptin concentration. On the final day of the food restriction phase (day 21), blood was collected from all rats in the hour preceding feeding (i.e., pre-prandial) and 10 min

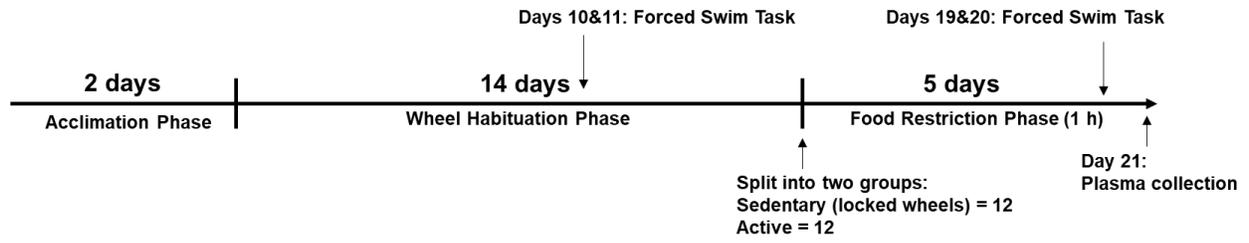


Figure 3.6. Timeline of experiment 1.2.

after feeding (i.e., post-prandial). A small cut was made at the tip of the tail and blood was collected in Eppendorf tubes which contained 20 µl of heparin per 1 ml of blood collected. Plasma was separated by centrifugation at 10,000 rpm for 10 minutes. Following centrifugation, blood plasma was aliquoted and stored at -80°C until processed using the ELISA kit.

Statistical Analysis

Running wheel activity. A 2 x 14 mixed ANOVA using *wheel condition* as the between-subject factor and *days* as the within-subjects factor was used to characterize wheel rotations across the 14 wheel habituation days. A 2 x 4 mixed ANOVA with *wheel condition* as the between-subject factor and *days* (the last 4 days of the wheel habituation phase) as the within-subjects factor was used to assess whether wheel rotations stabilised by the end of the wheel habituation phase. To examine the effect of food restriction on wheel rotations, a paired *t*-test was used comparing wheel rotations averaged across the last 4 days of the wheel habituation phase to the first 4 days of the food restriction phase.

Food intake. A paired *t*-test was used to compare food intake (g) averaged across the last 2 days of acclimation to the first 2 days of the wheel habituation phase. A 2 x 14 mixed ANOVA using *wheel condition* as the between-subject factor and *days* as the within-subjects factor was used to characterize food intake across the 14 wheel habituation days. A 2 x 4 mixed ANOVA with *wheel condition* as the between-subject factor and *days* (the last 4 days of the wheel habituation phase) as the within-subjects factor was used to assess whether food intake stabilised by the end of the wheel habituation phase. To examine the effect of food restriction on food intake, a paired *t*-test was used comparing food intake averaged across the last 4 days of the wheel habituation phase to the first 4 days of the food restriction phase. Finally, a 2 x 4 mixed ANOVA using *wheel condition* as the between-subjects factor and *days* as the within-subjects factor was used to characterise the change in food intake across the days of the food restriction phase.

Body weight. A paired *t*-test was used to compare body weight (g) averaged across the last 2 days of acclimation to the first 2 days of the wheel habituation phase. A 2 x 14 mixed ANOVA using *wheel condition* as the between-subject factor and *days* as the within-subjects factor was used to characterize body weight across the 14 wheel habituation days. A 2 x 2 mixed ANOVA was used with *wheel condition* as the between-subject factor and *phase* (body weight

on the last day of habituation versus the first day of food restriction) as the within-subjects factor. Finally, a 2 x 4 mixed ANOVA using *wheel condition* as the between-subjects factor and *days* as the within-subjects factor was used to characterise the change in food intake across the days of the food restriction phase.

Forced swim task. Time spent immobile, latency to immobility, and time spent climbing, in seconds, were noted for rats during the FST sessions. Three 2 x 2 mixed ANOVAs were used with each of the dependent variables using *wheel condition* as the between-subject factor and *phase* (wheel habituation phase versus food restriction phase) as the within-subject factor.

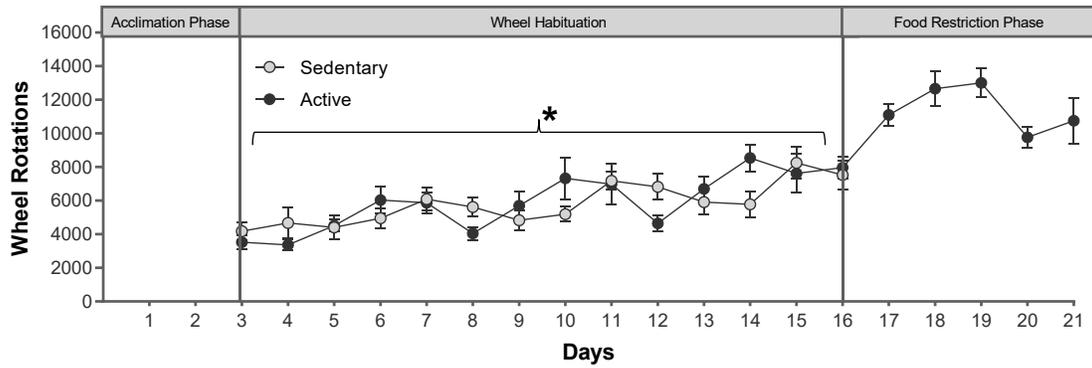
Plasma levels of leptin. A 2 x 2 mixed ANOVA using *wheel condition* as the between-subject factor and *phase* (preprandial versus post-prandial) as the within-subjects factor to characterize concentration of plasma leptin (ng/ml).

Results

Running wheel activity

There was a statistically significant gradual increase in running wheel activity across habituation days (*days*: $F(4.50, 99.08) = 8.49, p < .001, \eta_p^2 = .28$; Figure 3.7A). There was no statistically significant difference in running wheel activity between rats that would later be in the active condition versus those in the sedentary condition (*wheel condition*: $F(1, 22) = 0.03, p = .866, \eta_p^2 < 0.01$), and there was no statistically significant *days x wheel condition* interaction ($F(4.50, 99.08) = 2.34, p = .053, \eta_p^2 = 0.10$). Running wheel activity remained stable across the last 4 days of the habituation phase (*days*: $F(3, 66) = 1.76, p = .164, \eta_p^2 = 0.07$; *wheel condition*: $F(1, 22) = 1.36, p = .256, \eta_p^2 = 0.06$; *days x wheel condition*: $F(3, 66) = 1.65, p = .187, \eta_p^2 = 0.07$). As seen in Figure 3.7B, there was a statistically significant increase in the average of daily running wheel activity of rats in the active condition across the last 4 days of the habituation phase compared to the first 4 days of the food restriction phase ($t(11) = -7.20, p < .001, d = 2.08$).

A



B

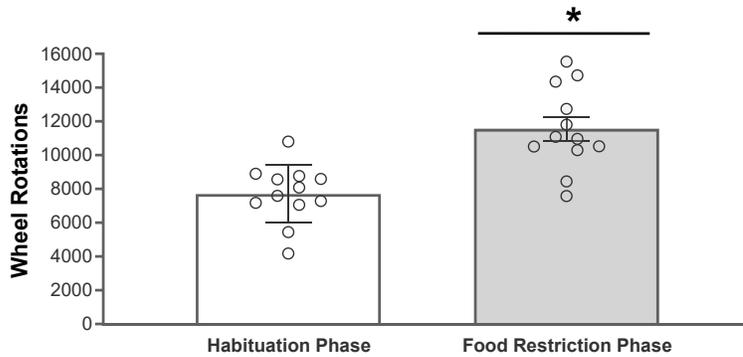


Figure 3.7. Effect of feeding schedule on running wheel activity. (A) Running wheel activity across experimental days, $*p < .001$. (B) Average running wheel activity across the last 4 days of the wheel habituation phase compared to the first 4 days of the food restriction phase, $*p < .001$.

Food intake

Average daily food intake was significantly higher during the first 2 days of the habituation phase compared to the 2 days of the acclimation phase ($t(23) = -3.73, p = .001, d = 0.76$; Figure 3.8A). Food intake showed a gradual and statistically significant increase across habituation days ($days: F(4.23, 93.11) = 13.39, p < .001, \eta_p^2 = 0.38$; Figure 3.8B). There was a statistically significant $days \times wheel\ condition$ interaction ($F(4.23, 93.11) = 2.91, p = .023, \eta_p^2 = 0.12$), but no main effect for $wheel\ condition$ ($F(1, 22) = 0.82, p = .376, \eta_p^2 = 0.04$). By the last 4 days of the habituation phase, food intake had stabilized ($days: F(1.92, 42.25) = 0.75, p = .472, \eta_p^2 = 0.03$), and there was no statistically significant main effect of $wheel\ condition$ ($F(1, 22) = 1.64, p = .213, \eta_p^2 = .07$), or $wheel\ condition \times days$ interaction ($F(1.92, 42.25) = 1.02, p = .366, \eta_p^2 = .04$).

Not surprisingly, daily food intake during the first 4 days of food restriction was significantly lower than food intake during the last 4 days of the habituation phase ($t(23) = 42.61, p < .001, d = 8.70$; Figure 3.8C). Food intake gradually increased across food restriction days ($days: F(3, 66) = 4.06, p = .010, \eta_p^2 = 0.16$). There were, however, no statistically significant differences between active and sedentary rats ($wheel\ condition: F(1, 22) = 0.48, p = .495, \eta_p^2 = .02$) and no significant $wheel\ condition \times days$ interaction ($F(3, 66) = 0.50, p = .682, \eta_p^2 = .02$; Figure 3.16B).

Body Weight

There was no statistically significant difference in body weight between the 2 acclimation days compared to the first 2 habituation days ($t(23) = 1.02, p = .319, d = 0.21$; Figure 3.9A). Across the 14 habituation days there was a gradual and statistically significant increase in body weight ($days: F(3.71, 81.63) = 14.01, p < .001, \eta_p^2 = 0.39$). There was no statistically significant main effect of $wheel\ condition$ ($F(1, 22) = 0.01, p = .929, \eta_p^2 < 0.01$) or $wheel\ condition \times days$ interaction ($F(3.71, 81.63) = 0.90, p = .463, \eta_p^2 = 0.04$; Figure 3.9B).

As expected, rats' body weight was significantly lower on the first day of the food restriction phase compared to that of the last day of the habituation phase ($phase: F(1, 22) = 643.35, p < .001, \eta_p^2 = .97$; Figure 3.9C). There was no statistically significant main effect of $wheel\ condition$ ($F(1, 22) = 0.01, p = .919, \eta_p^2 < 0.01$). There was, however, a statistically

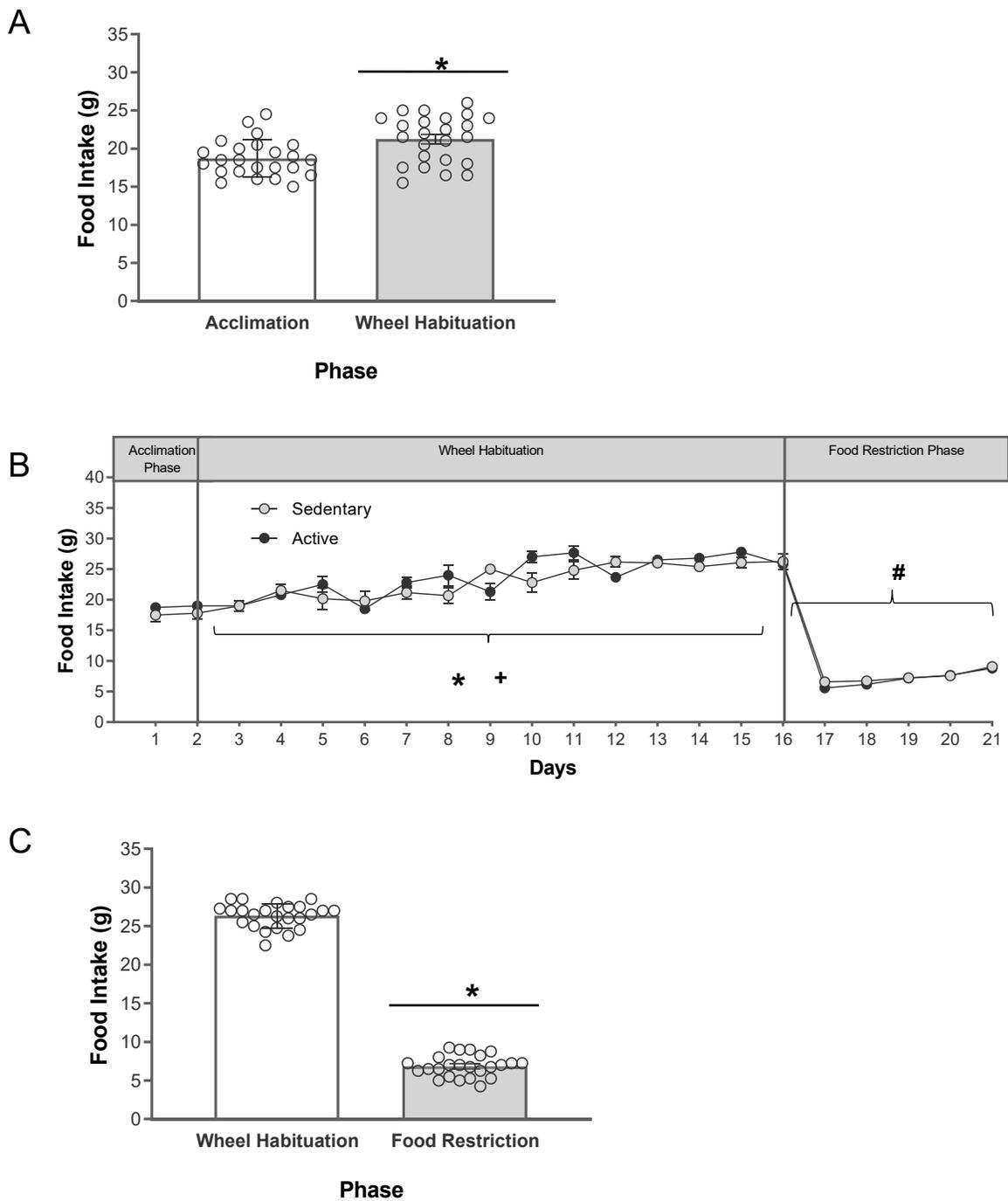


Figure 3.8. The effect of food restriction on daily food intake in sedentary and active rats. (A) Average daily food intake across the 2 days of the acclimation phase compared to the first 2 days of the wheel habituation phase, $*p = .001$. (B) Daily food intake across experimental days; $*p < .001$, main effect of *days*; $+p = .023$, *wheel condition x days* trending interaction; $\#p = .010$, main effect of *days*. (C) Average daily food intake across the last 4 days of the wheel habituation phase compared to the first 4 days of the food restriction phase, $*p < .001$.

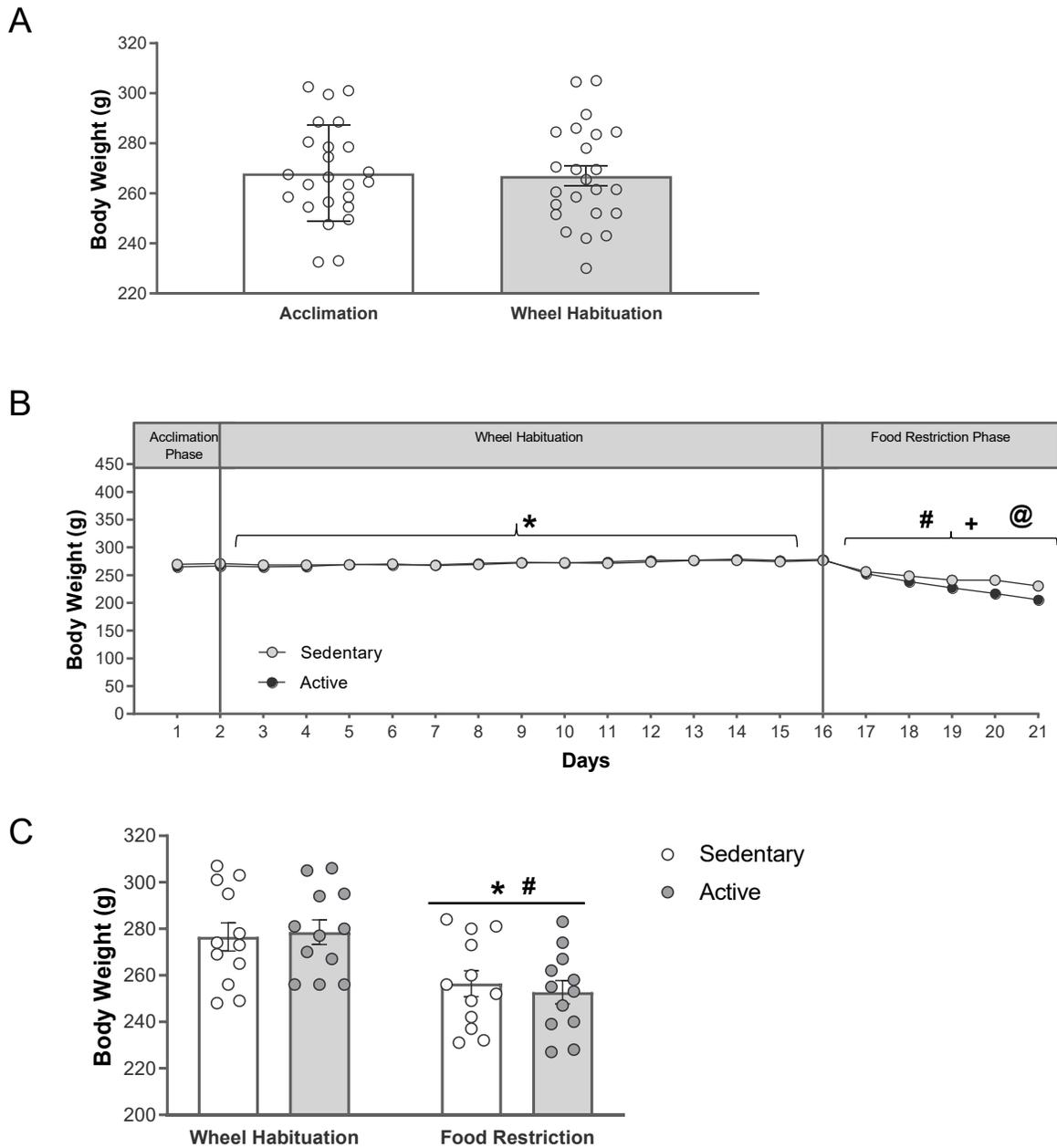


Figure 3.9. The effect of food restriction and running wheel activity on daily body weight in sedentary and active rats. (A) Average body weight on the two days of the acclimation phase compared to the first two days of the wheel habituation phase. (B) Daily body weight across experimental days, $*p < .001$; $\#p < .001$, main effect of *days*; $+p = .050$, trending main effect of *wheel condition*; $@p < .001$, *wheel condition* \times *phase* interaction. (C) Body weight on the last day of the wheel habituation phase compared to the first day of the food restriction phase, $*p < .001$, main effect of *phase*; $\#p < .001$, *wheel condition* \times *phase* interaction.

significant *wheel condition x days* interaction indicating that active rats weighed less than sedentary rats after the first day of the food restriction phase but not on the last day of the habituation phase ($F(1, 22) = 10.09, p < .001, \eta_p^2 = 0.31$). Across the 5 days of the food restriction phase, there was a gradual and statistically significant decrease in body weight, (*days*: $F(1.76, 38.74) = 108.84, p < .001, \eta_p^2 = 0.83$). The *weight condition* main effect was not statistically significant ($F(1, 22) = 4.28, p = .050, \eta_p^2 = 0.16$). There was, however, a statistically significant *wheel condition x days* interaction indicating that active rats lost more weight than the sedentary rats as the food restriction phase progressed ($F(1.76, 38.74) = 11.77, p < .001, \eta_p^2 = 0.35$; Figure 3.9B).

Forced Swim Task

As seen in Figure 3.10, for all variables of interest (i.e., latency to immobility, immobility time, time spent climbing), there were no statistically significant differences between phase (wheel habituation vs food restriction), or between active and sedentary conditions, and there were no statistically significant interactions (latency to immobility: *Phase*: $F(1, 22) = 1.56, p = .225, \eta_p^2 = 0.07$, *wheel condition*: $F(1, 22) = 0.91, p = .352, \eta_p^2 = 0.04$, *wheel condition x phase*: $F(1, 22) = 1.51, p = .225, \eta_p^2 = 0.06$; Immobility time: *Phase*: $F(1, 22) = 1.26, p = .274, \eta_p^2 = 0.05$, *wheel condition*: $F(1, 22) = 0.64, p = .433, \eta_p^2 = 0.03$, *wheel condition x phase*: $F(1, 22) = 1.11, p = .303, \eta_p^2 = 0.05$; Climbing: *Phase*: $F(1, 22) = 2.19, p = .153, \eta_p^2 = 0.09$, *wheel condition*: $F(1, 22) = 2.07, p = .164, \eta_p^2 = 0.09$, *wheel condition x phase*: $F(1, 22) = 2.01, p = .170, \eta_p^2 = 0.08$).

Plasma levels of leptin

Of the plasma samples collected from the 24 rats, only those of 12 rats were used to conduct the ELISA ($n_{\text{sedentary}} = 6, n_{\text{active}} = 6$). The remaining 12 rats were excluded as a result of insufficient amount of plasma or compromised quality of plasma at one or both of the sample collection time points. Furthermore, levels of leptin in plasma were generally very low, at times undetectable, compared to quantities reported in similar experiments (see discussion; Exner et al., 2000; Verhagen et al., 2011). As such, the following results are to be interpreted with caution. As seen in Figure 3.11, plasma concentrations of leptin were significantly reduced during the post-prandial phase compared to the pre-prandial phase (*phase*: $F(1, 10) = 6.00, p = .034, \eta_p^2 = 0.38$). Plasma leptin concentration did not appear to be affected by access to the

running wheel (*wheel condition*: $F(1, 10) = 0.42, p = .529, \eta_p^2 = 0.04$) and there was no statistically significant *wheel condition x phase* interaction ($F(1, 10) = 0.01, p = .979, \eta_p^2 < 0.01$).

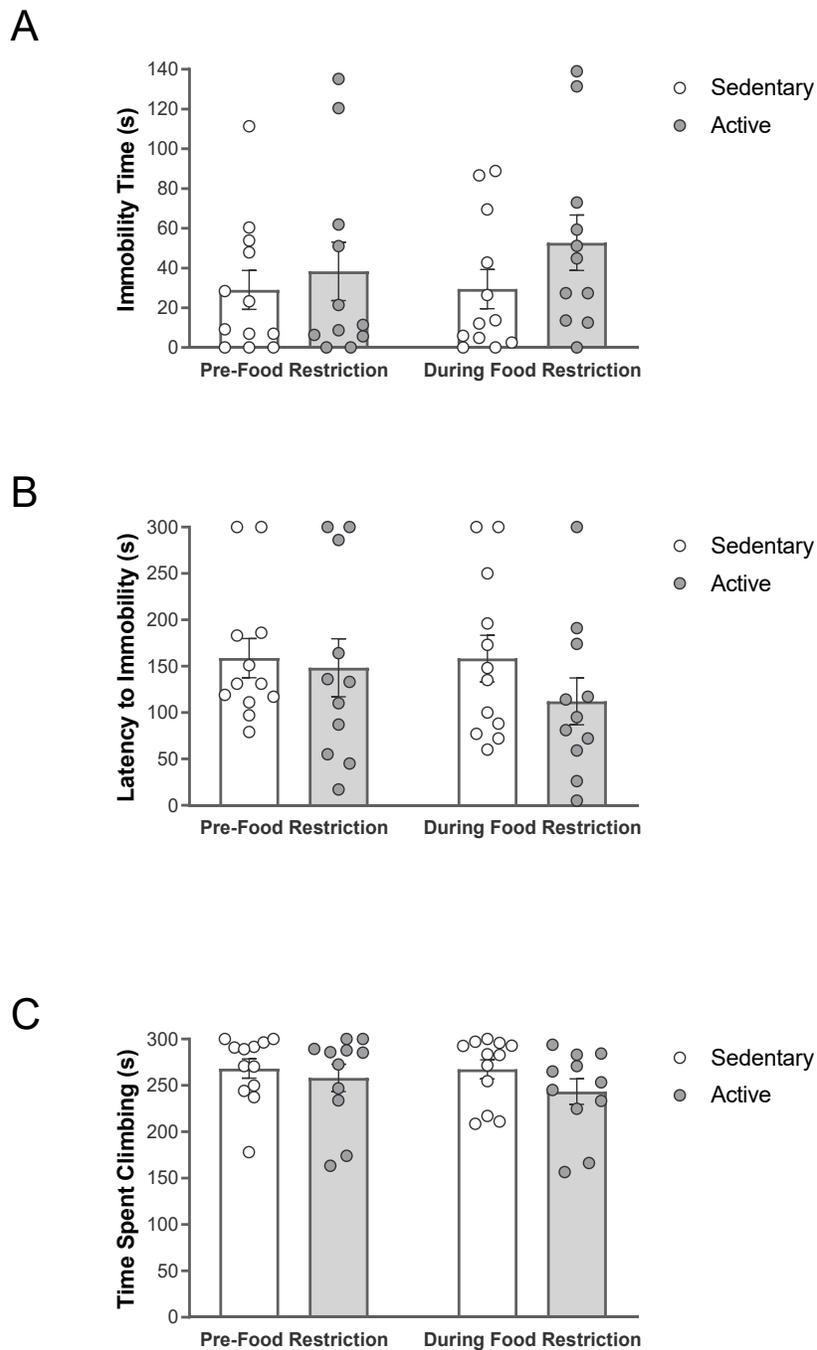


Figure 3.10. The effect of food restriction and wheel access on the FST. (A) Mean immobility time in seconds for sedentary and active rats pre-food restriction and during food restriction. (B) Mean latency to immobility in seconds for sedentary and active rats pre-food restriction and during food restriction. (C) Mean time spent climbing for sedentary and active rats pre-food restriction and during food restriction.

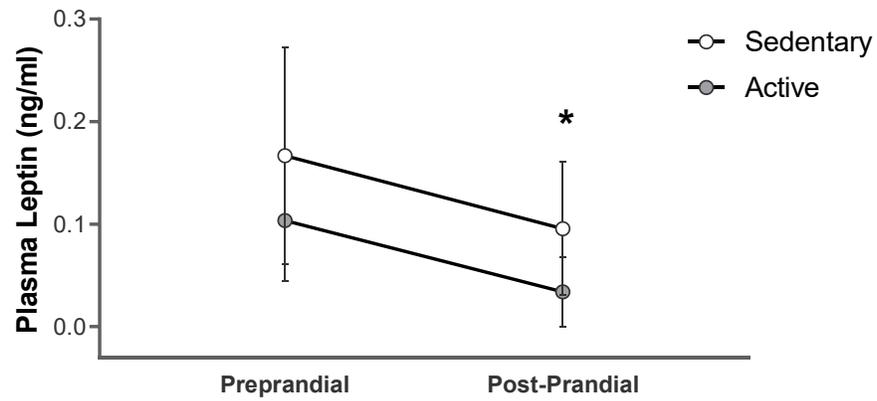


Figure 3.11. Concentration of plasma leptin in sedentary ($n = 6$) and active ($n = 6$) rats on the final day of the food restriction phase in the hour preceding feeding (preprandial) and 10 min after feeding (post-prandial), * $p = .034$, main effect of *phase*.

Discussion

The main goal of the present experiment was to examine the development of ABA in female Long-Evans rats. Having characterised ABA development in male Long-Evans rats in the three previous experiments, we were interested in using the same procedures in female rats to increase clinical relevance. Additionally, we sought to expand on the existing literature by examining the impact of ABA on depression-like behaviours and plasma leptin levels in female rats.

Wheel habituation phase

As expected, rats began using the running wheel on the first day of its introduction and showed a gradual increase in activity across days. Importantly, we found that the female rats in the present experiment were running at higher levels than those observed in male Long-Evans rats in the previous experiments. By the end of the wheel habituation phase, rats in the present experiment were running an average of 7287 wheel rotations a day compared to an average of 4648 wheel rotations a day run by the male rats in experiment 1.1. This observation is consistent with reports from ABA experiments reporting that female rodents tend to be more hyperactive overall than male rodents (Schalla & Stengel, 2019). Indeed, a wide variety of evidence suggests that female rodents exhibit elevated running rates relative to males, regardless of *ad libitum* or restricted feeding (Jones, Bellingham, & Ward, 1990; Lightfoot, 2013; Rosenfeld, 2017). One evolutionary explanation for this sex difference is that females may need to be more active or exploratory in searching for distant food sources needed to provide sufficient energy for themselves and their offspring (Lightfoot, 2013; Rosenfeld, 2017). Another more common explanation, though not necessarily mutually exclusive, is that sex differences in physical activity may be due to variations in sex hormones with increased estrogen and decreased testosterone in females (Lightfoot, 2013).

While we can confidently say that the female rats in the present experiment were more active than male rats from previous experiments in this chapter, it is unclear how their activity compares to those of females from other ABA experiments. Levels of wheel activity fluctuate greatly across female ABA experiments, possibly as a result of differences in rat strain and age. While the wheel habituation phase in the present experiment lasted 14 days, many of the ABA studies in females used a 7-day habituation phase. On the 7th day of the wheel habituation phase,

rats in the present experiment ran an average of 5270 daily wheel rotations. This is much higher than the approximate 2000 daily wheel rotations on the 7th day of the wheel habituation phase reported in adult Sprague Dawley females (Scharner et al., 2016). However, activity levels in adolescent female rats appears to be higher than those observed in the present experiment. For instance, Foldi, Milton, and Oldfield (2017) reported approximately 8000 daily wheel rotations on the 7th day of wheel habituation in adolescent Sprague Dawley rats. Adolescent Long-Evans rats were reported to run approximately 9000 daily wheel rotations on the 7th day of wheel pre-exposure (Kanarek, D'Anci, Jurdak, & Mathes, 2009).

Given the literature on wheel-induced feeding suppression in male rats and the observation of this phenomenon in experiments 1.1 and our preliminary study presented in Appendix 2, we had hypothesized that the introduction of the running wheel under sated conditions would result in a temporary reduction in food intake accompanied by acute weight loss. Our results did not support this hypothesis and instead showed an increase in food intake upon wheel introduction with continued weight gain. After further review of the literature on wheel-induced feeding suppression, it appears that our results do not necessarily contradict other findings. Wheel-induced feeding suppression is a phenomenon that has been predominantly examined in male rats (Afonso & Eikelboom, 2003; Belke & Dunbar, 1998; Lattanzio & Eikelboom, 2003; Routtenberg & Kuznesof, 1967). From the few studies that have examined the phenomenon in female rats, it appears that the effect of wheel introduction on food intake is much less consistent and apparent than in male rats. In a Master's thesis by Dalton-Jez (2006), the effect of wheel access on food consumption was examined in both males and females at different stages of development. While wheel-induced feeding suppression was found to have a clear pubertal onset in male rats, it only became apparent in female rats at PD 56 (i.e., early adulthood). Furthermore, females at all age groups showed a rapid compensation, whereby they increased their food intake relative to sedentary control rats which allowed them to maintain their body weight at or above that of control rats (Dalton-Jez, 2006). This is consistent with our finding that rats increased their food intake relative to the acclimation phase and continued to gain weight. ABA experiments in females have either not examined or not explicitly commented on wheel-induced feeding suppression prior to the onset of the food restriction phase. In one ABA experiment using Sprague Dawley adolescent females, rats given access to a wheel decreased their food intake briefly for two days before returning to the same amount as sedentary

control rats and there were no differences in body weight between the active and sedentary control rats (both groups continued to gain weight; Foldi et al., 2017). These results are consistent with our findings and those from Dalton-Jez (2006) that wheel-induced feeding suppression is not as consistent and severe in female rats compared to the effect reported in male rats.

Food restriction phase

As hypothesized, we observed a significant increase in running wheel activity in the active rats at the onset of food restriction. To confirm that this increase was truly the result of the change in feeding schedule rather than the passage of time, an active-sated control group would be needed. Nonetheless, rats' activity was stable across the last 4 days of the wheel habituation phase which suggests that the sudden increase in activity was in response to the beginning of food restriction. Our results also support the hypothesis that active rats would eat the same amount as sedentary rats during food restriction. Indeed, we found that, despite increased energy expenditure, active rats did not increase their food intake which resulted in accelerated weight loss compared to the sedentary rats. This is consistent with results from other ABA studies in females whereby active rats eat the same amount as their sedentary counterparts and are unable to compensate for increased energy expenditure (e.g., Allen, Jimerson, Kanarek, & Kocsis, 2017; Kanarek et al., 2009; Scharner et al., 2016). Importantly, we found that the ABA effect was stronger in female rats in that the starvation criterion was reached by the 5th day of the food restriction phase (compared to 7-8 days in males in the previous experiments in this chapter). This result is consistent with other ABA studies in female reporting a range of 3 to 7 days to reach starvation criterion (Cai et al., 2008; Giles, Hagman, Pan, MacLean, & Higgins, 2016; Kanarek et al., 2009; Verhagen, Luijendijk, de Groot, et al., 2011). The more rapid weight loss observed in female rats compared to male rats is likely the result of higher running wheel activity. A feeding time window of 90 min, instead of the 60 min used in the present experiment, may be more appropriate for female studies requiring longer survival time.

Forced Swim Test

Results of the FST did not support our hypotheses. Not only did we not observe any differences in immobility time or latency to immobility between active and sedentary rats during food restriction, we also did not observe any differences in FST behaviours from the wheel

habituation phase to the food restriction phase. To the best of our knowledge, this is the first experiment examining FST in female rats during ABA which complicates the interpretation of these null findings. Of course, one possible explanation for these findings is that food restriction simply does not result in depression-like behaviours in Long-Evans female rats. While this is possible, it would be inconsistent with studies that have shown that chronic or acute food restriction increases immobility time in male C57BL/6 mice and male Sprague Dawley rats (Alcaro, Cabib, Ventura, & Puglisi-Allegra, 2002; Jahng, 2011). On the other hand, another study using male Sprague Dawley rats found that a 24-hour food deprivation had no effect on FST behaviours (Abel, 1994). While FST is a simple and sensitive task, differences even in baseline immobility rates have been known to vary between different groups thereby complicating the comparison of results across studies (Bogdanova, Kanekar, D'Anci, & Renshaw, 2013). Certain rat strains are also known to develop more depressive-like behaviour in the FST and Long-Evans rats have been shown to show particularly contradictory results. For instance, chronic mild stress resulted in a greater increase in immobility in the FST in Long-Evans rats compared to Sprague Dawley rats (Bielajew et al., 2003). However, when the FST was carried out in a single 15-min session (i.e., without the habituation session), Long-Evans rats were less immobile than Sprague Dawley and Wistar rats (Abel, 1992). Given that there are no other studies examining FST during ABA in general and in female Long-Evans rats specifically, it will be worthwhile to repeat this test in a future experiment. To simplify interpretation during the ABA phase, it would be helpful to include sated control groups (e.g., a sedentary-sated group and an active-sated group) which would allow for examination of the effect of food restriction on FST.

Plasma Leptin

We found that plasma concentrations of leptin were reduced during the post-prandial phase compared to the pre-prandial phase and that it did not appear to be affected by access to the running wheel. These results, however, need to be interpreted with caution as the plasma leptin levels detected in the present experiment, averaging at 0.10 ng/ml, were atypically low. Plasma leptin levels are known to rapidly decrease in response to fasting so it is unsurprising that rats exposed to such a severe food restriction as the one used in the present experiment would result in low leptin concentration. While our results were consistent with one study that also

reported undetectable levels of plasma leptin in female Wistar rats exposed to ABA (Hillebrand, Van Elburg, et al., 2005) the levels observed in our experiment were lower than those typically reported in other ABA studies. For instance, Exner et al. (2000) reported an average plasma level of 0.75 ng/ml in male Wistar rats that had access to a running wheel and were fed 60% of their *ad libitum* food intake. This is particularly inconsistent with our results as leptin levels have been repeatedly shown to be significantly higher in female than in male rats, when equated for body weight (Pinilla et al., 1999; Wu-Peng, Rosenbaum, Nicolson, Chua, & Leibel, 1999). While the male rats in the Exner et al. (2000) study did weigh more (approximately 250 g) than the females in the present experiment (approximately 218 g) at the time of sample selection, the difference unlikely explains such a discrepancy in leptin concentration. (Verhagen, Luijendijk, de Groot, et al. (2011) reported levels ranging from 0.33 ng/ml to 0.50 ng/ml in female Wistar rats with continuous access to a running wheel while given food for 90 min/day. Contrary to what one may assume, the female Wistar rats weighed less (181-191 g) at the time of sample collection than the females in the present experiment. It is possible that strain differences may explain varying levels of plasma leptin concentration though there is little research on the topic. One study examining the effect of immobilization stress on plasma leptin levels in male and female Sprague Dawley and Long-Evans rats found that strain interacted with stress such that stressed Long-Evans rats displayed higher leptin levels than did stressed Sprague Dawley rats while no strain differences in leptin were observed in nonstressed animals (Ceballos, Faraday, & Klein, 2006). Thus, if we consider food restriction to be a stressor, we would assume that leptin concentrations in the Long-Evans rats in the present study would have been higher than that reported in other studies using food restriction in other rat strains. In addition to the concentrations being concerningly low, the effect that we did observe (lower leptin levels post-prandial compared to pre-prandial) is opposite to what is reported in the literature which suggests that refeeding following a fast results in an increase in circulating leptin (Ahrén, Månsson, Gingerich, & Havel, 1997; Boden, Chen, Mozzoli, & Ryan, 1996; Hardie, Rayner, Holmes, & Trayhurn, 1996; Weigle et al., 1997). Given the described discrepancies with the literature, no clear conclusions can be drawn based on these results.

Conclusions

This was the first experiment in the present dissertation using female rats. Consistent with the literature, we found that female rats generally ran more than the Long-Evans males in the previous experiments in this chapter. While the wheel-induced feeding suppression phenomenon was not observed in the present experiment, we did observe a robust ABA effect whereby rats increased their running wheel activity and failed to increase their food intake to compensate for the increased energy expenditure resulting in accelerated weight loss. It would be useful to include an active-sated control condition in future experiments to confirm that the increased activity was indeed due to the onset of food restriction. The ABA effect in the present experiment was so robust that rats reached the starvation criterion within 5 days. In future experiments, it would be worthwhile examining whether increasing the feeding window to 90 min (rather than 60 min used here) would allow rats to survive longer while preserving the ABA effect. We were surprised that food restriction or access to a wheel during food restriction (i.e., ABA) did not appear to affect depression-like behaviours on the FST. Given the known variability in FST and the fact that the present experiment was the first to examine FST in ABA in female rats, it would be advisable to repeat the FST procedure in future studies. Our plasma leptin results were inconclusive as the levels detected seemed concerningly lower than those reported in the literature.

EXPERIMENT 1.3. ACTIVITY-BASED ANOREXIA IN FEMALE LONG-EVANS RATS AND ITS EFFECTS ON PLASMA GHRELIN LEVELS

The previous experiment was the first in this dissertation examining the development of ABA in female Long-Evans rats. We found that female rats ran at higher levels than male rats in the previous experiments and showed a robust ABA effect whereby failure to increase their food intake during hyperactivity resulted in accelerated weight loss. In the present experiment, we aimed to build upon our findings in experiment 1.2 by including additional control conditions, expanding the feeding window to 90 min, and examining the effect of ABA on the “hunger hormone”, ghrelin.

In experiment 1.2, food restriction resulted in an increase in running wheel activity. In order to strengthen this claim that food restriction was inarguably resulting in hyperactivity, we included an active-sated control condition to the present experiment. We expected that food restricted rats would show a sharp increase in running wheel activity relative to the active-sated control rats.

In the previous experiment, we showed that adult female Long-Evans rats developed ABA so robustly that they reached starvation criterion within 5 days. Such a short survival window limits the tests that can be completed during acute ABA. In the present experiment, we hoped to prolong survival by allowing rats to feed for 90 min/day rather than 60 min used in experiment 1.2. Based on the work of other researchers who have used a 90 min feeding window in female rats (e.g., Foldi et al., 2017; Milton, Oldfield, & Foldi, 2018; Scharner et al., 2016; Scherma et al., 2017), we expected that active-food restricted rats, compared to the active-sated control rats, would show hyperactivity and accelerated weight loss (i.e., ABA) while maintaining their body weight above starvation criterion (75% of initial body weight) for more than 5 days.

Another goal of the present experiment was to investigate the effect of ABA on plasma levels of acylated ghrelin. Ghrelin is a peptide released from the gut that binds to the growth hormone secretagogue receptor (GHS-R1a) and, contrary to leptin, acts as a hunger signal (Kojima et al., 1999). Plasma levels of ghrelin spike prior to a meal and decrease after feeding in both humans (Cummings et al., 2001) and rodents (Toshinai et al., 2001). Plasma ghrelin levels have been shown to be elevated in individuals with AN (Méquinion et al., 2013) as well as in other chronic conditions of malnutrition (Legrand et al., 2016). In addition, ghrelin signaling and

modulation are impaired in AN as indicated by a delayed or absent postprandial decrease of ghrelin (Nedvídková et al., 2003; Stock et al., 2005). Rodent studies have indicated an important role of ghrelin in hyperactivity. For instance, peripheral ghrelin injections in Siberian hamsters increased foraging and hoarding behaviour (Keen-Rhinehart & Bartness, 2005) and ghrelin injections directly into the VTA of mice resulted in hyperactivity (Jerlhag et al., 2006, 2007). Ghrelin levels have been shown to increase over the course of ABA and to positively correlate with food anticipatory activity (FAA; Verhagen et al., 2010). Interestingly, GHS-R1a knockout mice show reduced FAA, indicating that activity can be reduced by suppressing ghrelin signaling (Verhagen et al., 2010). Similarly, FAA was suppressed when ABA rats were given an acute central injection of ghrelin receptor antagonist (Verhagen et al., 2010). Given the elevated ghrelin levels in response to food restriction and its role in hyperactivity, plasma ghrelin was measured in the present experiment pre-prandial and postprandial. We expected that food restricted rats would have higher plasma ghrelin than sated rats. Furthermore, we expected that ghrelin signaling would be disrupted in ABA, indicated by the absence of a reduction in ghrelin following feeding in active-food restricted rats.

Method

Subjects

Female Long-Evans rats ($n = 24$; 226-250 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec) and housed in a colony room on a 12:12 hr reverse light/dark cycle. Upon arrival, rats were initially pair-housed in plastic shoebox cages and left alone. On the following day (experimental day 1), rats were separated into individual shoebox cages allowing for baseline measures of daily food intake, water intake, and body weight. On day 5, rats were transferred to the laboratory where they were permanently housed in individual running wheel cages inside sound-attenuating boxes until the end of the experiment. At the onset of the food restriction phase (described in the procedure section below), rats were assigned to one of the following four conditions: sedentary-sated ($n = 6$), active-sated ($n = 6$), sedentary-FR ($n = 6$), or active-FR ($n = 6$). With the exception of the food restriction phase, rats had *ad libitum* access to both food and water throughout the experiment and body weight (g), food intake (g), and water intake (g) were monitored daily at ZT 11-12. Rats were removed from the experiment when they had lost 25% or more of their initial body weight.

Apparatus

Running Wheel Cages. See “General Methodology” section.

Plasma acylated ghrelin concentration. Plasma ghrelin was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Millipore, MA, USA). The reported detection sensitivity for the ELISA kit was 7.9 pg/ml.

Procedure

The detailed timeline is depicted in Figure 3.12.

Acclimation phase. Rats were individually housed in plastic shoebox cages for 4 days in the Animal Care Facility before being transported to the wheel cages. This period allowed for baseline daily measures of body weight, food intake, and water intake.

Wheel habituation phase. Following the 4 acclimation days, rats were transferred to the individual running wheel cages where they were permanently housed for the remainder of the experiment. During this phase, all rats had continued access to the running wheel and *ad libitum* food and water. This phase lasted 14 days (days 5-18).

Food restriction phase. At the onset of the food restriction phase, a metal rod was used to lock the running wheels of rats in the sedentary conditions while wheels of rats in the active conditions remained unlocked. Food was removed for rats in the food restricted conditions at ZT 13. On the following days, food restricted rats had access to food for 1.5 hr/day between ZT 12-13.5. Rats in the sated conditions continued to have *ad libitum* access to food. The experiment was terminated after 9 days of the food restriction phase.

Plasma acylated ghrelin concentration. On the third day of the food restriction phase (day 21), blood was collected from all rats in the hour preceding feeding (i.e., pre-prandial; approximately ZT 11) and 4 hours following feeding (i.e., post-prandial; approximately ZT 17.5). A small cut was made at the tip of the tail and blood was collected in Eppendorf tubes which contained 20 μ l of heparin per 1 ml of blood collected. Plasma was separated by centrifugation at 10,000 rpm for 10 minutes. Following centrifugation, blood plasma was aliquoted and stored at -80°C until processed. To protect the acylated ghrelin molecule, 1.0 μ l of

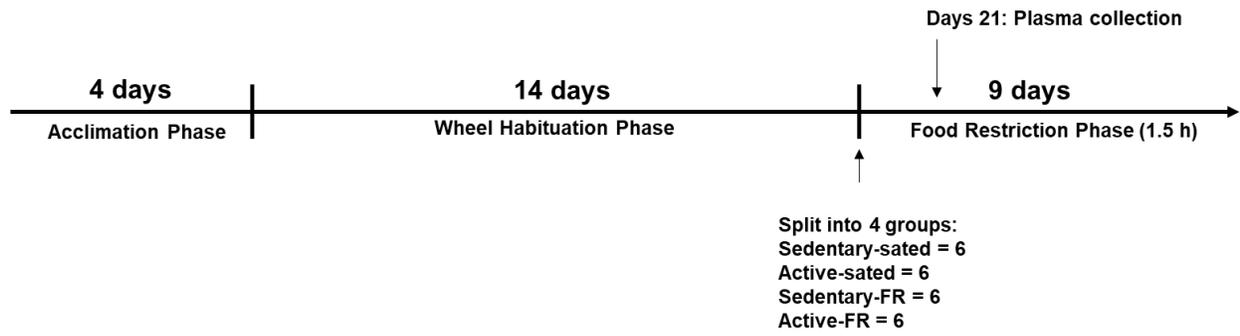


Figure 3.12. Timeline of experiment 1.3.

1.0 N HCl and 1.0 μ l of the protease inhibitor phenylmethylsulfonyl fluoride (Sigma-Aldrich) was added per 100 μ l of blood plasma.

Statistical Analysis

Wheel running, food intake, and body weight. A series of mixed ANOVAs were used to analyze each of the following dependent variables: wheel rotations, food intake, and body weight. Three (one for each independent variable) 4 x 14 mixed ANOVAs were used to characterize change across wheel habituation days. *Condition* (sedentary-sated, sedentary-FR, active-sated, active-FR) was used as the between-subjects factor and *days* (14 wheel habituation days) was used as the within-subjects factor. To examine if wheel activity, food intake, and body weight had stabilized by the end of the wheel habituation phase, three 4 x 5 mixed ANOVAs were used with *condition* as the between-subjects factor and *days* (5 last days of the wheel habituation days) was used as the within-subjects factor. To characterize the change from the wheel habituation phase to the food restriction phase, two (one for food intake and one for body weight) 4 x 2 mixed ANOVAs were used with *condition* (sedentary-sated, sedentary-FR, active-sated, active-FR) as the between-subjects factor and *phase* (average across the last 5 days of habituation compared to the average of the first 5 days of the food restriction phase) as the within-subjects factor. In the case of wheel rotations, a 2 x 2 ANOVA was used with only the active groups. To examine food intake during the food restriction phase, two 2 x 9 mixed ANOVAs were used with *days* (9 days of food restriction) as the within-subjects factor and *condition* (sedentary-sated vs. active-sated for the first analysis, sedentary-FR vs. active-FR for the second analysis). The same two ANOVAs were run with body weight as the dependent variable. For wheel rotations, the same analysis was run with only 2 levels of the *condition* factor (active-sated and active-FR).

Plasma levels of acylated ghrelin. To analyze plasma levels of ghrelin (pg/ml), a 2 x 2 x 2 mixed ANOVA was first conducted. *Wheel condition* (sedentary vs. active) and *feeding condition* (sated vs. food restricted) were used as between-subjects factors while *time* (preprandial vs. post-prandial) was used as the within-subjects factor. For ease of interpretation, two separate 2 x 2 mixed ANOVAs were also conducted. The first compared the sated conditions and the second compared the food restricted conditions. *Condition* was used as the between-subjects factor and *time* (preprandial vs. post-prandial) was used as the within-subjects factor.

Results

Running wheel activity

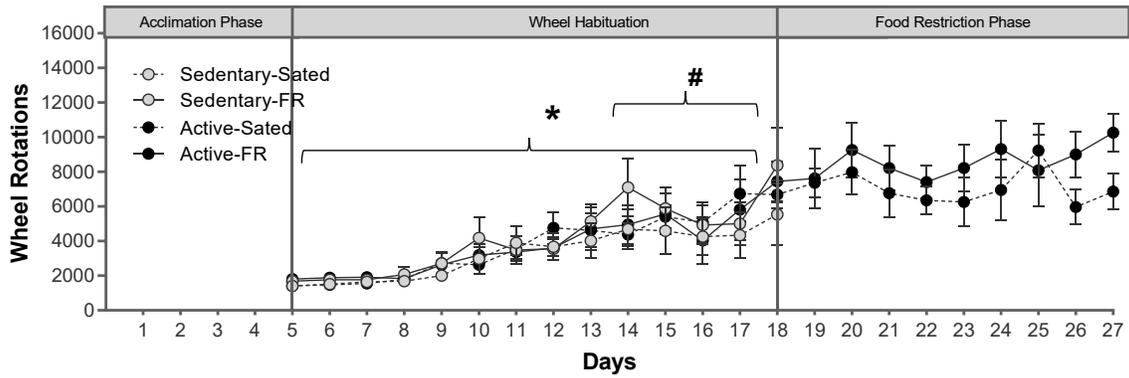
There was a statistically significant and gradual increase in running wheel activity across habituation days ($days: F(3.74, 74.79) = 27.28, p < .001, \eta_p^2 = 0.58$; Figure 3.13A). There was no statistically significant difference in running wheel activity between the eventual 4 conditions ($condition: F(1, 20) = 127.78, p = .854, \eta_p^2 = 0.04$), and there was no statistically significant $days \times wheel\ condition$ interaction ($F(3.74, 74.79) = 0.77, p = .669, \eta_p^2 = 0.10$). Although rats were given 14 days to habituate to the running wheel, running wheel activity continued to increase across the final 5 days of the habituation phase ($days: F(4, 80) = 5.58, p = .001, \eta_p^2 = 0.22$; $condition: F(3, 20) = 0.34, p = .795, \eta_p^2 = 0.05$; $days \times condition: F(12, 80) = 1.08, p = .385, \eta_p^2 = 0.14$).

As seen in Figure 3.13B, running wheel activity was significantly higher during the food restriction phase compared to the wheel habituation phase ($phase: F(1,10) = 34.36, p < .001, \eta_p^2 = 0.78$). There was no statistically significant difference between sated and FR conditions ($condition: F(1, 10) = 0.15, p = .707, \eta_p^2 = 0.02$). There was, however, a trending $phase \times condition$ interaction ($F(1, 10) = 3.86, p = .078, \eta_p^2 = 0.28$). There were no statistically significant differences in running wheel activity across the food restriction days and between the active-sated and active-FR rats ($condition: F(1, 10) = 0.95, p = .354, \eta_p^2 = 0.09$; $days: F(8, 80) = 1.15, p = .342, \eta_p^2 = 0.10$; $condition \times days: F(8, 80) = 1.28, p = .265, \eta_p^2 = 0.11$).

Food intake

Across the 14 days of the wheel habituation phase, food intake increased gradually ($days: F(13, 260) = 8.25, p < .001, \eta_p^2 = 0.29$) and there was no significant group differences or interaction ($condition: F(3, 20) = 0.42, p = .738, \eta_p^2 = 0.06$; $days \times condition: F(39, 260) = 0.66, p = .944, \eta_p^2 = 0.01$). While food intake did fluctuate across the last 5 days of the habituation phase, there was no statistically significant difference between conditions and no $days \times condition$ interaction ($days: F(2.59, 51.87) = 3.04, p = .044, \eta_p^2 = 0.13$; $condition: F(3, 20) = 0.75, p = .535, \eta_p^2 = 0.10$; $days \times condition: F(7.78, 51.87) = 0.31, p = .957, \eta_p^2 = 0.44$; Figure 3.14A).

A

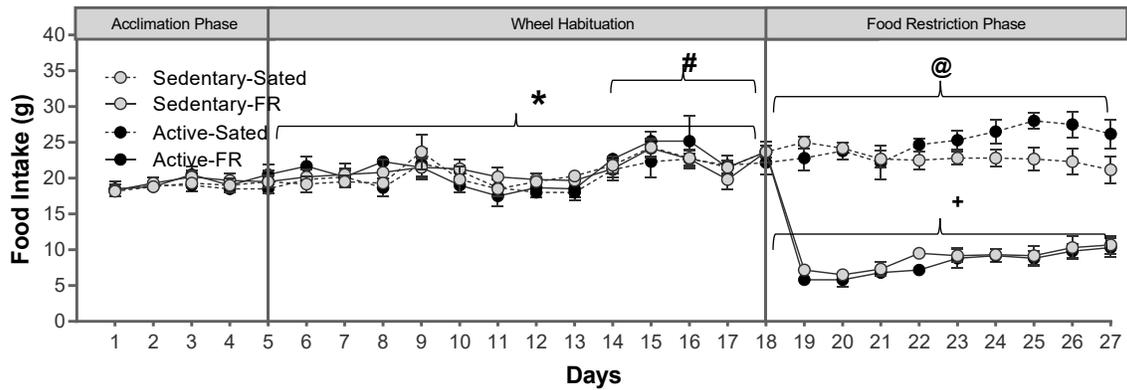


B



Figure 3.13. Effect of feeding schedule on running wheel activity. (A) Running wheel activity across experimental days; $*p < .001$, main effect of *days*; $\#p = .001$, main effect of *days*. (B) Average running wheel activity across the last 5 days of the wheel habituation phase compared to the first 5 days of the food restriction phase; $*p < .001$, main effect of *phase*; $+p = .078$, trending *phase x condition* interaction.

A



B

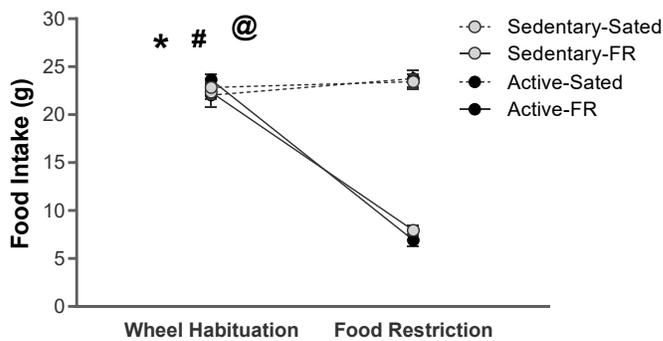


Figure 3.14. The effect of food restriction and wheel access on daily food intake. (A) Daily food intake across experimental days; $*p < .001$, main effect of *days*; $\#p = .044$, main effect of *days*; $@p = .031$, *days x condition* interaction; $+p < .001$, main effect of *days*. (B) Average daily food intake across the last 5 days of the wheel habituation phase compared to the first 5 days of the food restriction phase; $*p < .001$, main effect of *phase*; $\#p < .001$, main effect of *condition*; $@p < .001$, *phase x condition* interaction.

Not surprisingly, the start of the food restriction phase resulted in a statistically significant decrease in food intake compared to the daily food intake during the wheel habituation phase in both food restricted conditions but not in the sated conditions (*phase*: $F(1, 20) = 267.14, p < .001, \eta_p^2 = 0.93$; *condition*: $F(3, 20) = 51.33, p < .001, \eta_p^2 = 0.89$; *phase x condition*: $F(3, 20) = 121.69, p < .001, \eta_p^2 = 0.95$; Figure 3.14B).

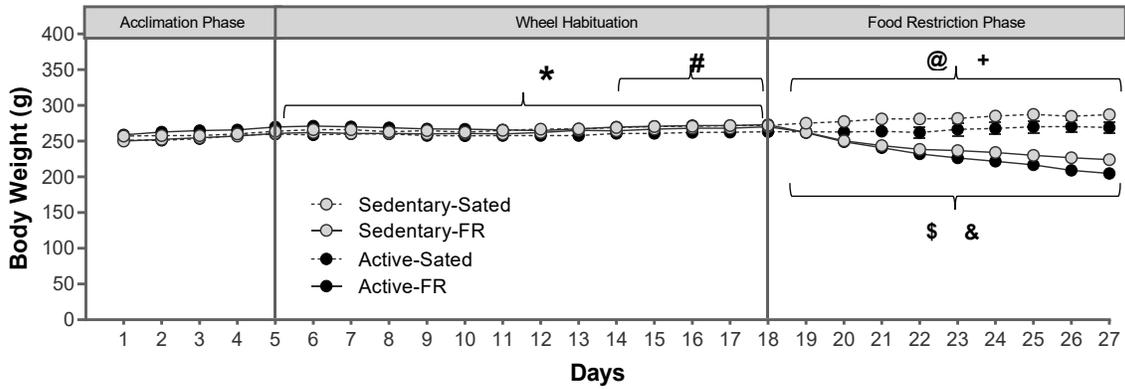
When comparing the sated conditions (sedentary - sated vs. active - sated) across the 9 days of the food restriction phase, there was no statistically significant main effect of *days* or *condition*, but there was a significant *days x condition* interaction indicating that rats in the active – sated condition gradually increased their food intake across days compared to the rats in the sedentary – sated condition whose food intake remained stable (*days*: $F(8,80) = 0.86, p = .553, \eta_p^2 = 0.08$; *condition*: $F(1, 10) = 3.68, p = .084, \eta_p^2 = 0.27$; *days x condition*: $F(8, 80) = 2.27, p = .031, \eta_p^2 = 0.19$; Figure 3.14A). This increase in food intake in active rats, presumably as an effort to compensate for increased energy expenditure, was not observed in the food restricted conditions. More specifically, when comparing the sedentary – food restricted condition to the active – food restricted condition, there was a significant main effect of *days*, but no significant main effect of *condition* or *days x condition* interaction (*days*: $F(8,80) = 6.41, p < .001, \eta_p^2 = 0.39$; *condition*: $F(1, 10) = 375.43, p = .426, \eta_p^2 = 0.06$; *days x condition*: $F(8, 80) = 0.32, p = .955, \eta_p^2 = 0.03$; Figure 3.14A).

Body weight

As seen in Figure 3.15A, body weight gradually increased across the 14 habituation days, but there were no group differences or *days x condition* interaction (*days*: $F(3.18, 63.65) = 8.11, p < .001, \eta_p^2 = 0.29$; *condition*: $F(1, 20) = 1.27, p = .311, \eta_p^2 = 0.16$; *days x condition*: $F(9.55, 63.65) = 0.69, p = .725, \eta_p^2 = 0.09$). Examination of body weight specifically across the final 5 days of the habituation phase indicated that body weight was continuing to increase at the end of this phase, again with no group differences or *days x condition* interaction (*days*: $F(4, 80) = 5.97, p < .001, \eta_p^2 = 0.23$; *condition*: $F(1, 20) = 1.10, p = .371, \eta_p^2 = 0.14$; *days x condition*: $F(12, 80) = 0.22, p = .997, \eta_p^2 = 0.03$).

The beginning of the food restriction phase resulted in significant changes in body weight and significant differences between conditions (*phase*: $F(1, 20) = 44.23, p < .001, \eta_p^2 = 0.69$; *condition*: $F(1, 20) = 3.35, p = .040, \eta_p^2 = 0.33$; Figure 3.15B). Importantly, there was a

A



B

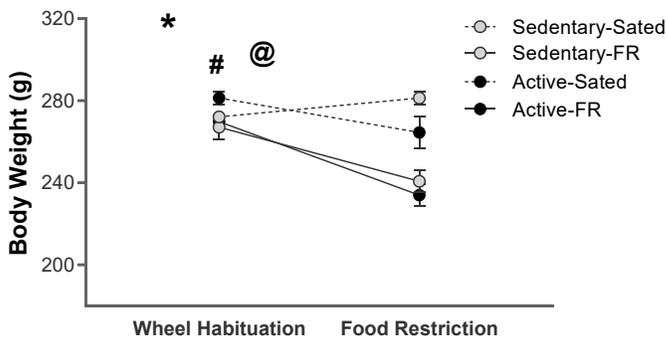


Figure 3.15. The effect of food restriction and running wheel activity on daily body weight. (A) Daily body weight across experimental days; $*p < .001$, main effect of *days*; $\#p < .001$, main effect of *days*; $@p < .001$, main effect of *days*; $+p = .080$, trending main effect of *condition*; $\$p < .001$, main effect of *days*; $\&p = .001$, *days x condition* interaction. (B) Average body weight across the last 5 days of the wheel habituation phase compared to the first 5 days of the food restriction phase; $*p < .001$, main effect of *phase*; $\#p = .040$, main effect of *condition*; $@p < .001$, *phase x condition* interaction.

statistically significant *phase x condition* interaction indicating that the change in body weight depended on the condition. More specifically, only rats in the food restricted conditions (sedentary-FR and active-FR) lost weight relative to the wheel habituation phase, while rats in the sedentary-sated condition gained weight and rats in the active-sated conditions maintained their body weight (*phase x condition*: $F(3, 20) = 35.78, p < .001, \eta_p^2 = 0.84$).

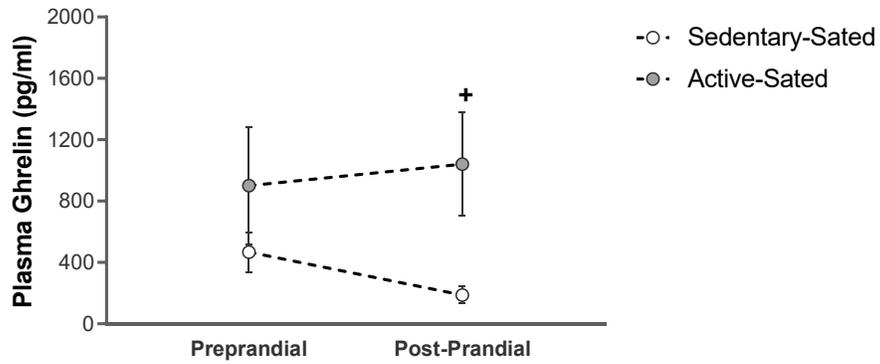
When comparing the sated conditions (sedentary-sated vs. active-sated) across the 9 days of the food restriction phase, there was a statistically significant main effect of *days*, and a trending main effect of *condition* that did not reach statistical significance (*days*: $F(3.90, 38.96) = 7.22, p < .001, \eta_p^2 = 0.42$; *condition*: $F(1, 10) = 3.81, p = .080, \eta_p^2 = 0.28$). There was no statistically significant *days x condition* interaction ($F(3.90, 38.96) = 0.71, p = .589, \eta_p^2 = 0.07$). When comparing the food restricted conditions (sedentary-FR vs. active-FR) across the 9 days of the food restriction phase, body weight decreased across the 9 days (*days*: $F(2.67, 26.69) = 148.59, p < .001, \eta_p^2 = 0.94$). While there was no significant main effect of *condition*, a statistically significant *days x condition* interaction indicated that rats in the active-FR condition showed a more rapid weight loss compared to rats in the sedentary-FR condition (*condition*: $F(1, 10) = 1.68, p = .225, \eta_p^2 = 0.14$; *days x condition*: $F(2.67, 26.69) = 7.53, p = .001, \eta_p^2 = 0.43$; Figure 3.15A).

Plasma levels of acylated ghrelin

Of the plasma samples collected from the 24 rats, only those of 13 rats were used to conduct the ELISA ($n_{\text{sedentary-sated}} = 4, n_{\text{active-sated}} = 3, n_{\text{sedentary-FR}} = 3, n_{\text{active-FR}} = 3$). The remaining 11 rats were excluded as a result of insufficient amount of plasma or compromised quality of plasma at one or both of the sample collection time points.

Plasma ghrelin levels are presented in Figure 3.16A and B. A three-way ANOVA revealed that ghrelin levels decreased from the pre-prandial phase to the post-prandial phase in the sedentary rats only (*time x wheel condition*: $F(1, 9) = 8.55, p = .017, \eta_p^2 = 0.49$). In addition, rats in the food restricted conditions had higher ghrelin levels than rats in the sated conditions (*feeding condition*: $F(1, 9) = 12.58, p = .006, \eta_p^2 = 0.58$). None of the other main effects and interactions reached statistical significance (*wheel condition*: $F(1, 9) = 2.72, p = .133, \eta_p^2 = 0.23$; *time*: $F(1, 9) = 1.42, p = .264, \eta_p^2 = 0.14$; *time x feeding condition*: $F(1, 9) = 0.31, p = .594, \eta_p^2 =$

A



B

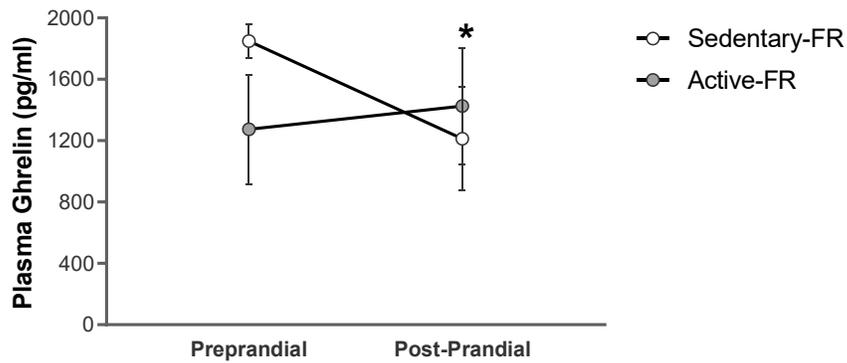


Figure 3.16. Preprandial and post-prandial concentration of plasma acylated ghrelin during the food restriction phase in (A) sedentary-sated and active-sated rats, $+p = .057$, trending main effect of *condition*, (B) sedentary-FR and active-FR rats, $* p = .041$, *time x condition* interaction.

0.03; *wheel condition x feeding condition*: $F(1, 9) = 2.87, p = .125, \eta_p^2 = 0.24$; *time x wheel condition x feeding condition*: $F(1, 9) = 4.65, p = .059, \eta_p^2 = 0.34$).

For ease of interpretation, two separate two-way ANOVAs were conducted. As seen in Figure 3.16A, when examining the stated conditions only (sedentary-sated vs. active-sated), active-sated rats appeared to have higher plasma ghrelin than sedentary-sated rats, though this difference did not reach statistical significance. There was no difference between pre-prandial and post-prandial and no *time x condition* interaction (*time*: $F(1, 5) = 2.31, p = .189, \eta_p^2 = 0.32$; *condition*: $F(1, 5) = 6.08, p = .057, \eta_p^2 = 0.55$; *time x condition*: $F(1, 5) = 0.45, p = .534, \eta_p^2 = 0.08$). However, when examining the food restricted conditions only (sedentary-FR vs. active-FR; Figure 3.16B), there was a statistically significant *time x condition* interaction ($F(1, 4) = 8.86, p = .041, \eta_p^2 = 0.69$) indicating that there was a difference in ghrelin plasma levels between the two collection time points in the sedentary-FR rats, but not in the active-FR rats. There was no statistically significant main effect of *time* ($F(1, 4) = 0.14, p = .728, \eta_p^2 = 0.03$) or *condition* ($F(1, 4) = 0.01, p = .978, \eta_p^2 < 0.01$).

Discussion

The main goal of the present experiment was to build upon our findings in experiment 1.2 with female Long-Evans rats by including additional control conditions and lengthening the feeding window to 90 min in an effort to prolong survival duration. Furthermore, we examined the effect of ABA on plasma concentration of ghrelin before and following feeding.

Wheel habituation phase

As observed in experiment 1.2, we found that the female rats in the present experiment also took to the wheel immediately upon introduction. Their running activity increased across days, reaching an average of 6952 daily wheel rotations on the last day of the wheel habituation phase. In fact, we found that, by the end of this phase, running wheel activity had not stabilized and instead was continuing to increase. Because the present experiment included an active-sated control condition, it was deemed unnecessary to wait until activity had stabilized before beginning food restriction. Our results, nonetheless, suggest that female rats, compared to the male rats in previous experiments, are more active and may require more days to reach a stable level of running wheel activity.

Food restriction phase

Our hypothesis that active-food restricted rats would show higher running wheel activity compared to the active-sated rats was only partially supported by a trending interaction. While there was an overall increase in activity from the wheel habituation phase to the food restriction phase, there was no significant difference in activity between the two conditions. The trending *phase x condition* interaction, however, was in the direction of our hypotheses whereby there were no group differences during the wheel habituation phase, but active-food restricted rats trended towards higher activity compared to active-sated rats during the food restriction phase. Nonetheless, because there was no statistically significant effect, we cannot determine whether the increase in activity over time was the result of food restriction (i.e., hyperactivity) or a progressive increase in activity that would have taken place regardless of food restriction until reaching a stable level.

We also found that the female rats in the present experiment were less active during the food restriction phase compared to the female rats in experiment 1.2, though they were more active than male rats in our previous experiments. In the present experiment, rats were fed *ad libitum* for 90 min/day while those in experiment 1.2 were fed for 60 min/day. Our results therefore suggest that the 90 min feeding window may not be severe enough to produce the hyperactivity effect that is so crucial to ABA.

The effect of the running wheel on food intake depended on whether rats were sated or food restricted. In the sated conditions, active rats compensated for their increased energy expenditure by increasing their food intake compared to the sedentary rats. In the food restricted conditions, however, there was no difference in food intake between the active and sedentary rats, indicating a failure to compensate for increased energy expenditure when activity and food restriction are combined. This finding was consistent with our previous results indicating that active rats under food restriction conditions do not necessarily eat less than sedentary rats who are also food restricted but instead do not increase their food intake to make up for the running activity, as is the case under sated conditions. This results in accelerated weight loss as was observed in the active-food restricted rats compared to the sedentary-food restricted rats. In experiment 1.2, with a 60-min feeding window, rats began to reach starvation criterion by the 5th day of the food restriction phase. Here, using a 90-min feeding window, only 1 rat had reached

starvation criterion by the 9th and final day of the food restriction phase. On average, active-sated rats weighed 78.24% of their initial body weight by the last day of the food restriction phase. In summary, using a longer feeding window allowed us to prolong survival while maintaining the accelerated weight loss in food restricted active rats versus sedentary rats. It is unclear, however, whether the hyperactivity effect was maintained.

Plasma levels of acylated ghrelin

Comparison of plasma levels of ghrelin between the two sated conditions (sedentary-sated and active-sated) revealed a trend for increased ghrelin in the active rats. There has been ample research suggesting that increased ghrelin signaling results in more voluntary exercise (Jerlhag et al., 2006, 2007). There has been less, however, on the effects of exercise, under sated conditions, on ghrelin levels. Our results, though not statistically significant, suggest that having access to a running wheel increases plasma ghrelin concentration. This finding would need to be replicated with a larger sample size.

The more interesting comparison here was that of the food restricted conditions (sedentary-FR and active-FR) indicating that severe food restriction seemed to have interacted with running wheel activity to interfere in the entrainment of feeding control signals. More specifically, we found that sedentary-FR rats showed the expected pre-meal peak in ghrelin levels and postprandial decrease while active-FR rats ghrelin concentrations did not shift from pre-prandial to post-prandial. This finding is consistent with reports from the human literature that individuals with AN show delayed or absent postprandial decreases of ghrelin (Nedvídková et al., 2003; Stock et al., 2005). This disruption in hunger signals in response to ABA may partly explain why ABA rats do not eat enough to survive and continue to run after a meal. Given the small sample size in the present experiment, these results would need to be replicated, ideally with a larger sample size. Furthermore, it would be interesting to collect plasma at various timepoints across the food restriction phase to obtain a better sense of changes in ghrelin levels from the beginning to the end of ABA.

Conclusions

In an effort to extend survival time in ABA, rats in the present experiment were given 90 min/day of food access. While rats in the active-FR condition continued to display accelerated

weight loss, this longer feeding window did result in longer survival with rats still not having reached the starvation criterion by the 9th and final day of the food restriction phase. The hyperactivity effect, however, was less robust with this extended feeding window, suggesting that the 90 min/day feeding schedule not be as effective in producing the ABA effect as is the 60 min/day feeding schedule in female Long-Evans rats. Our preliminary results of plasma ghrelin concentration suggested that severe food restriction may interact with running wheel activity to interfere with hunger signals in ABA.

EXPERIMENT 1.4. EFFECT OF ABA ON ATTENTIONAL SET-SHIFTING IN FEMALE LONG-EVANS RATS

In the previous experiments, we demonstrated that female Long-Evans rats, under conditions of food restriction and unlimited wheel access, reliably develop ABA. In the present experiment, we aimed to build on our findings by examining the effect of ABA development on cognitive flexibility.

Individuals with AN have been characterized by perfectionism, behavioural rigidity, and ritualized behaviours concerning eating, weight, and shape. Indeed, the over-importance of the control of weight and shape in AN leads to ritualistic behaviours such as extreme dietary restriction and excessive physical exercise that are compulsive in nature. Not surprisingly, OCD, a disorder known for its compulsivity, and AN often co-occur (Godart et al., 2006; Kaye et al., 2004) and share genetic etiologies (Watson et al., 2019; Yilmaz et al., 2020). One domain of compulsivity is cognitive flexibility which refers to the ability to shift between multiple tasks, operations, or mental sets (Roberts et al., 2007). This set-shifting ability allows for the modification of cognitive strategies in novel or uncertain contexts that is necessary to adapt to changing demands (Milton et al., 2020). Clinically, cognitive flexibility is most commonly assessed using tasks such as the Wisconsin Card Sorting Test (WCST), the Trail Making Task, or the Brixton Task. Set-shifting impairments in these tasks, defined as more perseverative errors, have consistently been reported during acute phases of AN (Wu et al., 2014) as well as in recovered individuals (Lindner et al., 2014). Furthermore, cognitive inflexibility has also been demonstrated in healthy sisters of patients with AN (Roberts et al., 2010)

At the time the present experiment was conducted, cognitive flexibility had not been examined in the context of ABA. Behavioural paradigms designed to assess cognitive flexibility in rodents require elaborate training and are therefore time-consuming. Given that ABA develops rapidly and can result in rats reaching starvation criterion within as little as 4 days, the logistics of testing ABA rats in such an elaborate behavioural paradigm may have deterred researchers. In the present experiment, we aimed to take on this challenge by training rats in a maze-based cognitive flexibility procedure prior to beginning the ABA procedure. Different rodent behavioural paradigms of cognitive flexibility assess distinct components of this construct: set-shifting (i.e., cross-modal shifting or extradimensional shifting) involves inhibiting responses

based on one dimension that was previously correct and learning to respond based on a different dimension, while reversal learning (intramodal shifting or intradimensional shifting) refers to the ability to inhibit a response to one exemplar in a particular dimension and learning to respond to another exemplar within the same dimension (Dias, Robbins, & Roberts, 1996, 1997). As the clinical literature has been most consistent in reporting deficits in set-shifting in individuals with AN, we adapted a “behavioural flexibility task” described by Ragozzino, Detrick, and Kesner (1999) to assess set-shifting ability during ABA. In this maze-based task, rats are first taught the correct response in a given dimension (e.g., turning left to obtain a food reward in the “response dimension”). After response acquisition, rats are asked to “set-shift” by learning a response in a new dimension (e.g., turning in the black arm of the maze to obtain a food reward in the “visual cue dimension”). Slower acquisition of this new rule reflects poorer set-shifting ability. Based on the increased perseveration in individuals with AN in human tests of cognitive flexibility, we hypothesized that rats exposed to ABA, compared to sedentary control rats, would display set-shifting deficits as indicated by longer acquisition time and more perseverative errors.

Method

Subjects

Female Long-Evans rats ($n = 12$; 226-250 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec) and housed in a colony room on a 12:12 hr reverse light/dark cycle. Upon arrival, rats were initially pair-housed in plastic shoebox cages and left alone. On the following day, rats were separated into individual shoebox cages and handled daily for an additional 3 days. After this 4-day acclimation period, rats were transferred to the laboratory where they were permanently housed in individual running wheel cages inside sound-attenuating boxes until the end of the experiment. At the onset of the ABA phase (described in the procedure section below), rats were assigned to one of the following conditions: sedentary ($n = 6$) or active ($n = 6$). Throughout the experiment, body weight (g), food intake (g), and water intake (g) were monitored daily at ZT 11-12. Rats were removed from the experiment when they had lost 25% or more of their initial body weight.

Apparatus

Running Wheel Cages. See “General Methodology” section.

Cognitive Flexibility Maze. Cognitive flexibility was assessed using a plus-maze based set-shifting procedure. The plus-maze walls extended 28 cm above a wire grid flooring which measured 10.5 cm in width. The roof of the maze was transparent. Four arms were arranged at 90° angles around a 14 x14 cm central chamber and measured 75 cm in length. Black polyurethane guillotine doors were used to open or close the entrance to the arms and to the goal arms from the central chamber. At the end of each arm was a metal food recipient. One of the arms was panelled with white plastic serving as a visual cue. The maze was placed inside a dark room equipped with red lights.

Procedure

The timeline for the present experiment can be seen in Figure 3.17. The experiment consisted of the following 5 phases which are described in detail below: wheel acclimation, maze habituation, training, recovery, and ABA. During all phases, rats were housed in their individual running wheel cages and moved to and from the maze room when needed. Maze procedures were carried out between ZT 12-16 and the time remained consistent for each rat throughout.

Wheel acclimation phase. Four days following arrival, rats were transferred from the animal care facility to the individual running wheel cages where they were permanently housed for the remainder of the experiment. During this phase, all rats had *ad libitum* food and water and continuous access to the running wheel. This phase lasted 4 days.

Maze habituation phase. The maze habituation procedure began following the 4 days of wheel acclimation. In an effort to increase rat motivation to work in the maze, rats were mildly food restricted (90% of initial body weight) during this phase and the subsequent phase (training phase described below). On day 1 of the maze habituation phase, three Froot Loops were placed in each arm, two of them in the food well at the end of the arm. Rats were allowed to move freely in the maze for 15 min. If all 12 Froot Loops were eaten in less than 15 min, the rat was placed in the yellow holding bucket while the arms were re-baited before it was placed back into the maze for the remaining time. On day 2, the same procedure as day 1 was repeated, however, when the rat ate two Froot Loops, it was removed from the maze and placed into another arm. This was done in an effort to acclimatize the rats to being handled after eating the cereal reward.

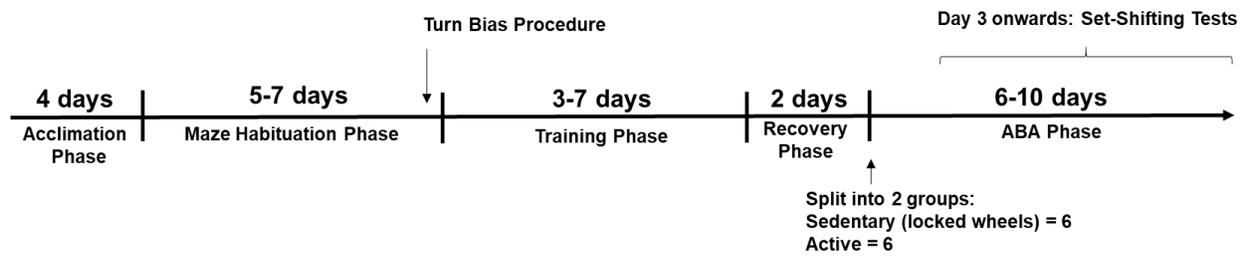


Figure 3.17. Timeline of experiment 1.4.

The subsequent habituation sessions were the same as on day 2 except only two half loops were placed in the food wells only. This procedure continued daily until the rat consumed the rewards from all food wells for four trials in a 15 min session. One trial consisted of the rat consuming all the rewards in every arm. As such, the duration of this phase varied across rats and ranged between 5 and 7 days.

Turn-bias procedure. On the final day of the maze habituation phase, rats were tested to establish their turn bias. The maze was turned into a t-maze by blocking off one of the arms. The rat was placed at the beginning of the start arm and allowed to turn left or right to obtain a half Froot Loop from the food well at the end of the arm (both arms were baited). Once the rat turned and consumed the reward, it was picked up, placed in the same start arm, and allowed to make another choice of arm. If the rat chose the same initial arm, it was returned to the starting arm until it chose the opposite arm from its initial choice and ate the cereal. After choosing both arms, the rat was returned to the yellow holding bucket, the block and visual cue were moved to different arms and a new trial began. The turn bias trial consisted of entering both choice arms and consuming the cereal for a total of 7 trials. Each initial turn was recorded and summed up at the end of the 7 trials to provide a turn-bias (4 or more turns in the same direction out of 7). This turn bias was later used for the response-discrimination testing where the rat was trained to turn the opposite direction of its turn bias.

Training Phase. During the training phase, rats started from one of the arms designated west, south, or east. One of the arms had white paneling which served as a visual cue and was placed in different arms pseudo-randomly. For every consecutive set of 12 trials, the visual cue appeared an equal number of times in each arm. Rats had to either learn the response discrimination rule or the visual-cue discrimination rule. The rule learned was counterbalanced across the sedentary and active conditions. For response discrimination, the rat was placed in a starting arm (never north) and had to learn to turn in the opposite direction of its turn-bias in order to reach the food well with a half-piece of Froot Loops. For visual-cue discrimination, the rat had to learn to turn into the arm with the visual cue to obtain the reward. This procedure was repeated for 24 trials daily until the rats successfully completed 10 consecutive trials. Once this was achieved, the rat was tested in a probe phase, where the rat started from the north arm, which had never been used before to avoid possible use of visual cues in the room. If the rat correctly

followed the same rule as on training, the training phase was complete. However, if the rat made an incorrect turn, training continued as described above until the rat made 5 consecutive correct choices at which point another probe trial was administered. This procedure continued until the probe was successfully completed and ranged between 3-7 days.

Recovery Phase. Once rats completed the training phase, they were allowed to rest and recover for 2 days. During these 2 days, rats were taken off the mild food restriction used to train the rats in the maze and instead were given *ad libitum* food. They remained in their running wheel cages during these two days.

Activity-Based Anorexia Phase. Following the two days of recovery, body weight and running wheel activity were used to match rats into the sedentary or active condition. Rats in the active condition continued to have continuous access to their running wheel while the wheels of the rats in the sedentary condition were locked in place. Food was removed for all rats at ZT 13. On the following days, rats had access to food for 1 hr/day between ZT 12-13. The set-shifting test began on the 3rd day of the ABA phase and was conducted within this phase.

Set-Shifting Test. On the 3rd day of the ABA phase, the set-shifting test was initiated and, as previously, rats were transported to the maze room in the yellow bucket and immediately returned to their running wheel cages following the maze procedure. During the set-shifting procedure, rats had to ignore the previously relevant rule (response discrimination or visual-cue discrimination) in order to acquire a new rule. Rats that had previously been trained in response discrimination were now trained on visual cue discrimination and vice versa. The same procedure as described above in the training phase was implemented here where the rat had to achieve 10 correct trials consecutively and a final probe.

Statistical Analysis

Wheel rotations, food intake, and body weight. To characterize running wheel activity across the experiment, a mixed 2 x 4 ANOVA was used with wheel rotations as the dependent variable. The between-subjects factor was *condition* (active or sedentary) and the within-subjects-factor was *phase* (wheel acclimation, maze habituation, training, and recovery). A paired *t*-test was used to compare average wheel rotations during the two days of recovery and the first 3 days of the ABA phase. Two 2 x 2 mixed ANOVAs were used for the dependent

variables of food intake and body weight. For each, *condition* served again as the between-subjects factor and *phase* (recovery vs. ABA phase) as the within-subjects factor.

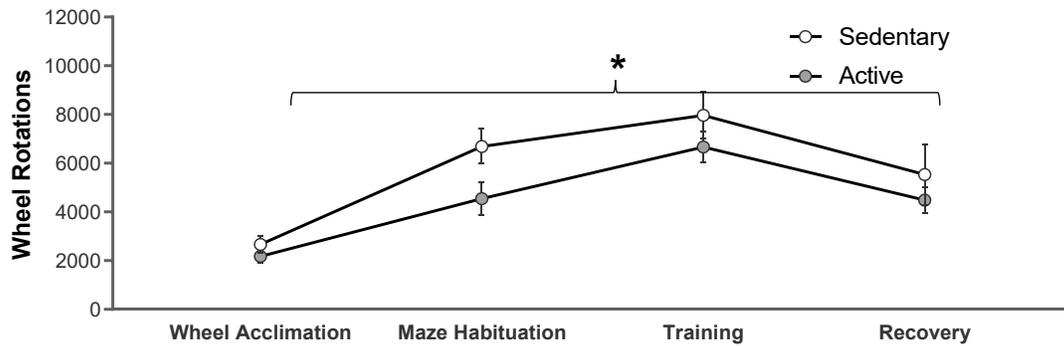
Set-Shifting. Independent samples *t*-tests were conducted to compare the active condition to the sedentary condition on the following measures: (1) acquisition (the number of trials required for the rat to reach its first probe test); (2) criterion (number of trials until the rat successfully completed the probe); (3) probes (number of probes completed until correct response on probe test); and (4) null trials (number of trials during which the rat did not move for 5 consecutive min). These variables were analyzed for both the pre-shift and post-shift phases of the task. In addition, independent samples *t*-tests were conducted to compare both conditions on errors committed during the post-shift phase. Errors, as described by Block, Dhanji, Thompson-Tardif, and Floresco (2007) included: (1) never-reinforced errors (rat follows neither of the rules learned during response or visual cue discrimination); (2) regressive errors (rats are assumed to have learned the new rule, but sporadically revert back to the previous rule); and (3) perseverative errors (rats continue to use the previously learned rule when the trial requires turning the opposite direction). Perseverative errors were counted by dividing the trials into blocks of four trials and when the rat used the first acquired rule three times or more within one block, it was considered to be perseverative. When the rats used the first rule less than three times out of four within one block, the errors were counted as regressive.

Results

Running wheel activity

As seen in Figure 3.18A, there was a statistically significant change in running wheel activity across the first 4 phases of the experiment with no statistically significant main effect of *wheel condition* or *phase x wheel condition* interaction (*phase*: $F(3, 30) = 17.84, p < .001, \eta_p^2 = 0.64$; *wheel condition*: $F(1, 10) = 4.10, p = .070, \eta_p^2 = 0.29$; *phase x wheel condition*: $F(3, 30) = 0.51, p = .678, \eta_p^2 = 0.05$). Pairwise comparisons with a Bonferroni correction revealed a significant increase in running activity from the wheel acclimation phase to the maze habituation phase ($p < .001$), training phase ($p < .001$), and recovery phase ($p = .049$). There were no statistically significant changes in running activity between the habituation, training, and recovery phases. As seen in Figure 3.18B, food restriction resulted in a statistically significant

A



B

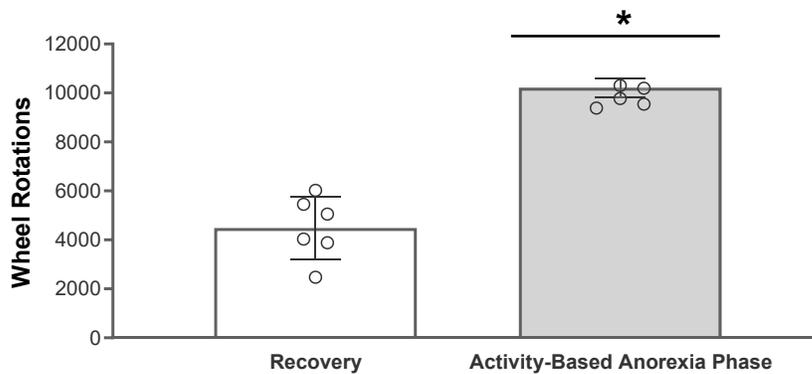


Figure 3.18. Running wheel activity across the different experimental phases. (A) Average running wheel activity across the various phases of the experiment in rats that were later in the sedentary condition compared to rats who were later in the active condition; $*p < .001$, main effect of *phase*. (B) Average wheel running activity of active rats during the recovery and ABA phases of the experiment; $*p < .001$, compared to recovery.

increase in running wheel activity (for rats in the active condition) during the ABA phase compared to the recovery phase ($t(5) = 9.49, p < .001, d = 3.87$).

Food intake

As expected, food restriction during the ABA phase resulted in a statistically significant decrease in food intake compared to food intake during the recovery phase (Figure 3.19A). Importantly, there was no statistically significant main effect of *wheel condition* and no *phase x wheel condition* interaction (*phase*: $F(1, 10) = 614.08, p < .001, \eta_p^2 = 0.98$; *wheel condition*: $F(1, 10) = 0.93, p = .357, \eta_p^2 = 0.09$; *phase x wheel condition*: $F(1, 10) = 0.64, p = .442, \eta_p^2 = 0.06$).

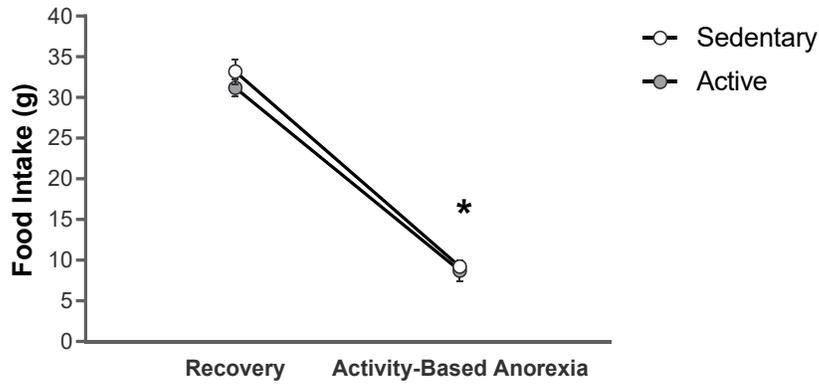
Body weight

Food restriction during the ABA phase resulted in a statistically significant decrease in body weight compared to that of the recovery phase. While there was no statistically significant main effect of *wheel condition*, there was a statistically significant *phase x wheel condition* interaction indicating accelerated weight loss in rats in the active condition during the ABA phase (*phase*: $F(1, 10) = 305.50, p < .001, \eta_p^2 = 0.97$; *wheel condition*: $F(1, 10) = 0.33, p = .580, \eta_p^2 = 0.03$; *phase x wheel condition*: $F(1, 10) = 7.96, p = .018, \eta_p^2 = 0.44$, Figure 3.19B).

Cognitive Flexibility

There were no statistically significant differences between sedentary and active rats in any of the variables of interest during the training phase or during the set-shifting test that took place during the ABA phase (Table 3.1).

A



B

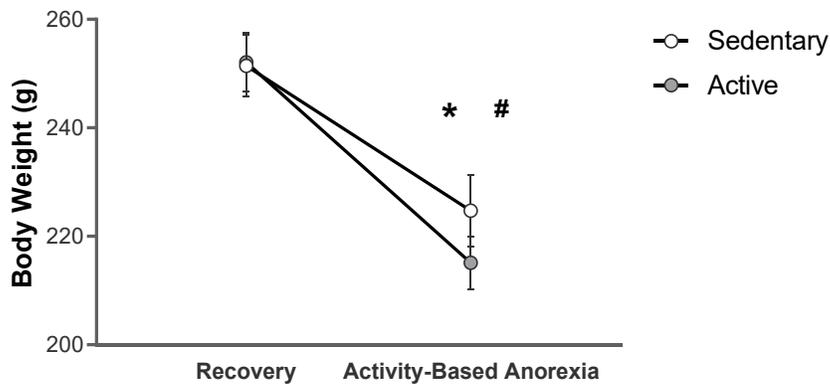


Figure 3.19. The effect of food restriction and wheel access on daily food intake. (A) Daily food intake across experimental days; $*p < .001$, main effect of *phase*. (B) Average daily food intake across the last 5 days of the wheel habituation phase compared to the first 5 days of the food restriction phase; $*p < .001$, main effect of *phase*; $\#p = .018$, *condition x phase* interaction.

Table 3.1.*T-Test Results on Set-Shifting Variables of Interest*

	Sedentary	Active	<i>t</i>	<i>p</i>	<i>d</i>
	<i>M (SEM)</i>	<i>M (SEM)</i>			
Training Phase					
Acquisition Criterion	68.50 (12.22)	97.33 (12.26)	1.67	0.127	0.96
Trials to Criterion	93.33 (11.54)	97.33 (12.26)	0.24	0.817	0.14
Set-Shifting Test					
Acquisition Criterion	97.17 (13.13)	105.67 (11.63)	0.49	0.638	0.28
Trials to Criterion	110.50 (9.10)	105.67 (11.63)	-0.33	0.750	0.19
Perseverative Errors	12.67 (4.19)	7.67 (3.88)	-0.88	0.401	-0.51
Regressive Errors	14.17 (2.24)	16.33 (3.18)	0.56	0.590	0.32
Never-Reinforced Errors	9.00 (2.79)	7.50 (2.16)	-0.43	0.680	0.25

Note. N = 12; df = 10

Discussion

Individuals with AN have been reported to show deficits in cognitive flexibility (Wu et al., 2014). Yet, at the time the present experiment was conducted, cognitive flexibility had not been assessed in the context of ABA. The present experiment was designed to assess attentional set-shifting ability in female rats exposed to ABA. It was hypothesized that rats exposed to ABA, compared to sedentary control rats, would make more perseverative errors indicating deficits in cognitive flexibility.

ABA Development

ABA developed as expected and in a way that was consistent with our previous experiments. More specifically, we found that food restriction significantly increased running wheel activity. As reported in previous experiments, we again found that active rats did not eat more than sedentary rats suggesting that their failure to compensate for increased energy expenditure explains the accelerated weight loss in these rats, relative to the sedentary control rats. Importantly, we found that daily training in the set-shifting procedure did not interfere with habituation to the running wheel or to the development of ABA.

Effect of ABA on attentional set-shifting

Our hypothesis that ABA would result in deficits in cognitive flexibility (as indexed by perseverative errors) was not supported. In fact, no statistically significant differences were found between active and sedentary control rats on any of the variables of interest.

While our results failed to support our hypotheses, they appear to be consistent with the current literature. Indeed, since the completion of the present experiment, two papers have been published on cognitive flexibility in ABA. Similarly to our experiment, one group examined cognitive flexibility in female Sprague Dawley rats exposed to ABA compared to weight-paired control rats (Allen et al., 2017). The authors used a digging attentional set-shifting task with odor and digging medium as dimensional sets. Importantly, their procedure assessed for reversal learning (e.g., reward previously associated with lemon scent now associated with almond scent, regardless of digging medium) in addition to set-shifting (e.g., reward that was previously associated with a specific scent is now associated with a specific digging medium - for example,

marbles - regardless of scent). Consistent with our findings, Allen et al., (2017) did not find set-shifting impairment as a result of ABA, though they did report impairments in reversal learning.

To this date, the only other study examining cognitive flexibility in ABA is a recent publication by Milton et al. (2020) that set out to investigate the neurobiological link between weight loss in ABA and cognitive flexibility. Using designer receptors exclusively activated by designer drugs (DREADDs) to selectively modulate neurons projecting from the medial PFC to the NAcc shell, the authors found that suppression of this corticostriatal circuit allowed rats to maintain their body weight during ABA and prevented perseverative responding in a touchscreen cognitive flexibility task. They concluded that the parallel between weight loss and cognitive flexibility in ABA aligns with the relationship between disrupted prefrontal function and cognitive rigidity observed in AN. Importantly, however, the cognitive flexibility task used by Milton et al. (2020) assessed reversal learning, but not set-shifting. Taken together, the evidence seems to suggest that if ABA does result in impairments in cognitive flexibility in female rats, it occurs via deficits in reversal learning, but not necessarily set-shifting.

This conclusion may come as a surprise given the numerous reports of impaired set-shifting in humans with AN. It would appear, however, that what has often been referred to as “set-shifting” in studies of AN has been a misnomer for the broader multidimensional construct of cognitive flexibility. Indeed, in a brief publication highlighting this very problem, Wildes, Forbes, and Marcus (2014) explain that research on cognitive flexibility in eating disorders have relied heavily on multidimensional neuropsychological measures such as the WCST and have used perseverative errors on such tasks as an index of cognitive inflexibility. Due to the WCST’s complexity, however, perseverative errors indicating cognitive inflexibility can reflect disruptions in two discrete neurocognitive processes: reversal learning and/or attentional set-shifting. These two processes have distinct neural correlates with reversal learning depending on intact orbitofrontal cortex functioning while set-shifting relies on the medial PFC (Bissonette, Powell, & Roesch, 2013). Therefore, the use of behavioural tasks designed to assess specific cognitive processes, rather than neuropsychological tests assessing multidimensional constructs, could help advance the understanding of cognitive inflexibility in AN.

Although clinical studies in AN examining reversal learning ability separately from set-shifting are scarce, one study found that low-weight adolescents with AN performed more poorly

on a probabilistic object reversal task compared to healthy controls (Sarrar et al., 2011). The CANTAB intra- /extra-dimensional task is a tool that effectively distinguishes set-shifting ability from reversal learning. Our finding that ABA did not result in impaired set-shifting ability is consistent with three clinical studies that found no significant deficits in set-shifting in low weight individuals with AN using the intra- /extra-dimensional task (Fowler et al., 2006; Galimberti, Martoni, Cavallini, Erzegovesi, & Bellodi, 2012; McAnarney et al., 2011). It is therefore possible that the cognitive inflexibility observed in AN results from deficits in reversal learning rather than set-shifting. Future studies investigating cognitive flexibility in ABA would benefit from using behavioural paradigms that effectively distinguish between these two neurocognitive processes.

Another potential explanation as to why we did not observe deficits in set-shifting ability during ABA has to do with the acuteness of the ABA effect. In an effort to explain why they observed deficits in reversal learning but not in set-shifting during ABA, Allen et al. (2017) make the argument that reversal learning and set-shifting deficits may develop at different times over the course of AN. Indeed, one of the limitations of the ABA model is that it models acute excessive weight loss and hyperactivity but fails to model the chronic and relapsing nature of AN. Although not much is known about the time course of cognitive impairment in AN, it is possible that reversal learning deficits are prominent earlier in the course of AN while deficits in set-shifting may develop more gradually and become more prominent at more advanced stages of illness (Allen et al., 2017). The ABA paradigm used in the present experiment is likely more suitable for modeling early phases of weight loss. Future studies examining cognitive flexibility in ABA may want to assess reversal learning and set-shifting at different time points and using a chronic version of ABA.

Finally, it is possible that our failure to observe set-shifting impairments in ABA resulted from limitations with our set-shifting procedure. For instance, the mild food restriction during the training phase (when all rats had access to the running wheel) may have obscured eventual differences between the two groups of rats. In other words, both conditions were exposed to a “mild” form of ABA whereby they had access to the running wheel activity while being food restricted to 90% of their initial body weight. This may have led to a deterioration in set-shifting ability in all rats prior to even beginning the food restriction phase. Future designs would benefit

from including a control condition that never has access to the running wheel. Another limitation of our design was the use of food as a reward in the set-shifting procedure. While the rats were severely food restricted during the ABA phase, they did have access to extra calories during the daily set-shifting task which may have slowed the ABA effect and again obscured potential differences between the active and sedentary rats. This is not a simple fix as most behavioural tests of cognition in rodents use some form of food as a reward. An option to be considered in future designs would be to submerge the maze into a pool of water, similar to the pools used in the Morris Water Maze, where the rats would be motivated to learn the fastest way to an elevated platform rather than to a food reward. A water procedure may also address the issue of motivation whereby rats regularly lost motivation to work for the cheerios, sometimes having to complete 24 trials in one session. Allen et al. (2017) discuss this same issue whereby subjects in a pilot experiment did not remain motivated to dig for cheerios for enough trials to complete the task. They resolved this issue by using ground chow presented in small (0.1 g) pieces.

CHAPTER 3 SUMMARY

The overarching goal of the experiments presented in this chapter was to determine whether we could establish the ABA model in adult Long-Evans rats in our laboratory. In two preliminary studies (presented in Appendix 1 and 2), we investigated the effect of pre-exposure to the feeding schedule and to the running wheel on the development of ABA. The subsequent experiments presented here investigated ABA development in male and female rats on various feeding schedules. We also began to test the feasibility of using behavioural tests, such as the FST and a maze-based set-shifting task, in conjunction with the ABA paradigm.

Given our preliminary findings (see Appendix 1 and 2) that sedentary male rats were unable to maintain their body weight under a 60 min/day feeding schedule and that rats exposed to this schedule and concurrent wheel access rapidly met starvation criterion (within 7 days), *experiment 1.1* was designed to examine ABA development using a 90 min/day feeding schedule in Long-Evans male rats. A sedentary control condition was also added to examine the effect of wheel access on food intake during food restriction. Finally, changes in depression-like behaviour in response to ABA were assessed using the FST which also allowed us to determine whether behavioural testing could be used in the context of ABA without interfering with its development. Despite the longer feeding time, rats showed a robust ABA response whereby they increased their activity upon food restriction, ate the same amount as the sedentary control rats and thus showed accelerated weight loss reaching starvation criterion within 8 days. Unfortunately, due to baseline differences in FST between the two conditions, we were unable to determine if and how ABA impacted FST. FST testing did not, however, impact ABA development indicating the feasibility of using such procedures in conjunction with the ABA paradigm.

Given the higher prevalence rate of AN in women, the main goal of the remaining experiments in this chapter was to characterize ABA in female Long-Evans rats. In *experiment 1.2*, we demonstrated a robust ABA effect in female rats exposed to a strict restricted feeding schedule of 60 min/day food access. Consistent with our previous findings in male rats and results from other studies in female rats, we found that food restriction resulted in hyperactivity and a failure to adjust food intake accordingly, resulting in accelerated weight loss. This ABA effect was so robust that rats reached starvation criterion within 5 days. ABA did not result in

depression-like behaviour in the FST, but we again demonstrated the feasibility of using such a behavioural task without interfering with ABA development. In an effort to extend survival time in ABA, rats in *experiment 1.3* were given 90 min/day of food access. While rats in the ABA condition continued to display accelerated weight loss, this longer feeding window did result in longer survival with rats still above starvation criterion by the 9th and final day of this ABA phase. The hyperactivity effect, however, was less obvious with this extended feeding window, suggesting that the 90 min/day feeding schedule not be as effective in producing the ABA effect as is the 60 min/day feeding schedule in female Long-Evans rats. Finally, *experiment 1.4* was our first attempt at assessing the effect of ABA on cognitive functioning, and more specifically set-shifting ability. We found that we were able to carry out a time-consuming maze-based set shifting procedure, involving lengthy training, without interfering with the development of ABA. Contrary to our hypotheses, however, we did not find ABA to impact set-shifting ability. Possible explanations for these results include the use of “set-shifting deficits” in the human literature as a misnomer for general cognitive inflexibility which may be driven by impairments in reversal learning rather than set-shifting, time course of cognitive impairment in AN and ABA, and limitations of our set-shifting procedure. These potential explanations are discussed in further detail in the discussion section of this experiment.

An interesting and serendipitous finding that emerged from this series of experiments was the large amount of individual variability in running wheel activity in rats exposed to the ABA procedure. In both male and female experiments, we found that a subset of rats, when exposed to ABA, tended to dramatically increase their running wheel activity resulting in rapid weight loss while another subset of rats showed a more modest increase in activity or managed to increase their food intake to compensate for their running resulting in slower weight loss. The large range in running wheel activity during in each experiment reflects this individual variability in response to ABA. As the experiments presented in this chapter, most ABA studies have been designed to draw comparisons between rats exposed to ABA and control conditions (e.g., sedentary or sated), but few have examined differences between ABA-resilient and ABA-susceptible rats. Closer examination of the differences between these subsets of rats may help shed light into susceptibility factors relevant for the human condition of AN.

**CHAPTER 4: RESILIENCE AND SUSCEPTIBILITY TO ABA IN FEMALE SPRAGUE
DAWLEY RATS**

ABSTRACT

In Chapter 3, we observed important individual differences in response to the activity-based anorexia (ABA) model. Upon simultaneous food restriction and wheel access, some rats adapted by either increasing their food intake or decreasing their running wheel activity allowing them to maintain their body weight (i.e., resilient rats). Under the same conditions, other rats seemed to increase their running wheel activity and fail to increase their food intake which resulted in accelerated weight loss (i.e., susceptible rats). The goal of the experiments presented in this chapter was to further the understanding of ABA susceptibility by comparing resilient and susceptible rats on measures of anxiety- and depression-like behaviours, mesolimbic DA, and response to treatment with the DA antagonist, olanzapine (OLZ). All experiments were conducted in adult female Sprague Dawley rats. In experiment 2.1 we tested for trait differences in the forced swim test (FST), elevated-plus maze (EPM), and the sucrose preference test (SPT) prior to the onset of ABA. While no differences were observed in the EPM and SPT, susceptible rats showed longer latency to immobility and less immobility time in the FST compared to resilient rats. In experiment 2.2, locomotor activity following amphetamine (AMPH) challenges was used as an index of mesolimbic DA activity to test whether differences in this system before and after ABA are associated with ABA susceptibility. No differences were observed suggesting that any differences in mesolimbic DA between resilient and susceptible rats that may exist during ABA are not present at baseline. These findings also suggest that the increased baseline wheel activity in susceptible rats is not driven by differences in mesolimbic DA. In experiment 2.3, rats were exposed to two bouts of ABA. Between the two bouts, rats were implanted with subcutaneous osmotic minipumps allowing for chronic infusion of OLZ (7.5 mg/kg/day) or vehicle. OLZ reduced running wheel activity in both resilient and susceptible rats. We observed that there was less variability in ABA response during the second bout of ABA which complicated trait differences. The results from these experiments are individually interpreted in their respective discussions and further discussed in the context of recent publications in the general discussion (Chapter 7).

CHAPTER INTRODUCTION

Given the severity of the health consequences and high mortality rate of AN, identification of at-risk populations prior to the onset of disease would be advantageous. Yet, little is known about the aetiology and risk factors of AN. Identifying predictive factors of AN could enable early detection, help in prevention efforts, and increase treatment efficacy. Unfortunately, prospective longitudinal studies are difficult and scarce due to the young age of potential study participants, the low prevalence of the disorder, and the many years of follow-up required (Kaye, Fudge, & Paulus, 2009). Animal models, such as ABA, can be particularly helpful in filling this gap. One promising approach is to examine resilience and susceptibility in ABA. In chapter 3 we observed varying levels of susceptibility in rats exposed to the ABA procedure. More specifically, some rats (“susceptible”) experienced rapid weight loss in response to ABA resulting in accelerated experimental exit while other rats (“resilient”) either maintained their weight or showed a more gradual weight loss when exposed to ABA. The overarching goal of the studies presented in this chapter was to further characterize this resilience and susceptibility trait. To facilitate the ease of comparison across studies, we opted to use female Sprague Dawley rats as they are most commonly used in ABA studies. Experiments in this chapter aimed to assess for the presence of behavioural and neurobiological differences. We also aimed to determine whether susceptibility could be reduced through pharmacological treatment.

Despite the many ABA studies that have emerged since the onset of the model, little is known about resilience and susceptibility to ABA. In more recent years, researchers have been paying more attention to individual differences in ABA and their potential contributing factors. Large variability in weight loss during ABA has been reported between mice strains suggesting genetic risk (Gelegen et al., 2010, 2008; Gelegen et al., 2007; Pjetri et al., 2012). For instance, Gelegen et al. (2007) found that C57BL/6 mice housed with running wheels and exposed to a restricted feeding schedule reduced their wheel activity in contrast to DBA/2J mice who increased their activity under these same conditions. In addition, while both mouse strains experienced hypoleptinemia during ABA, the authors found that the decline in plasma leptin was stronger in DBA/2J mice which may contribute to increased susceptibility. It is also possible that

DBA/2J mice's stronger susceptibility to ABA may be related to this strain's higher level of anxiety and exploratory behaviour compared to C57BL/6 mice (Crawley et al., 1997).

One of the most consistent predictors of ABA susceptibility has been the level of baseline activity under *ad libitum* feeding conditions. In attempting to identify a susceptible subtype to ABA, Barbarich-Marsteller et al. (2013) found that rats with maximal hyperactivity and minimal food intake were the most susceptible while rats with minimal activity were more resilient. Higher baseline running wheel activity has repeatedly been shown to be related to greater weight loss during ABA in both mice and rats (Barbarich-Marsteller et al., 2013; Perez-Leighton, Grace, Billington, & Kotz, 2014; Pjetri et al., 2012). Perez-Leighton et al. (2014) found that baseline spontaneous physical activity (voluntary activity in a cage without a wheel) predicted baseline running wheel activity and ABA susceptibility. By examining the microstructure of running wheel activity in rats exposed to ABA, Wu et al. (2014) found that rats with the most severe weight loss during ABA had the lowest level of FAA and that postprandial activity (PPA) was more directly predictive of weight loss.

The goal of the experiments presented in this chapter was to further contribute to the understanding of ABA susceptibility by investigating different avenues. In experiment 2.1, we examined whether baseline differences in anxiety-like and depression-like behaviours exist between resilient and susceptible rats. In experiment 2.2, locomotor activity following amphetamine (AMPH) challenges was used as an index of mesolimbic DA activity to test whether differences in this system before and after ABA are associated with ABA susceptibility. Finally, in experiment 2.3, we examined the effect of chronic administration of an atypical antipsychotic medication, OLZ, on ABA development in resilient and susceptible rats.

EXPERIMENT 2.1. BASELINE DIFFERENCES IN ANXIETY- AND DEPRESSION-LIKE BEHAVIOURS BETWEEN RESILIENT AND SUSCEPTIBLE RATS

The high comorbidity between AN and depressive and anxious symptoms is well recognized and associated with lower body mass index and higher AN symptoms (Godart et al., 2006, 2015; Godart et al., 2007; Guarda, Schreyer, Boersma, Tamashiro, & Moran, 2015). Importantly, depressive and anxious symptoms are present not only during acute phases of AN but have also been shown to occur before illness onset and to persist after recovery (Godart et al., 2015; Guarda et al., 2015). This would suggest that these symptoms are not merely a consequence of malnutrition but instead may represent predisposing factors for AN. There have been no studies, however, examining whether baseline depressive-like and anxiety-like behaviours are predictive of ABA susceptibility in rats.

A key depressive symptom of AN is anhedonia whereby patients are unable to derive normal pleasure associated with reward. For instance, individuals recovered from AN show aversion to energy-dense foods (Jiang, Soussignan, Rigaud, & Schaal, 2010). They have also been reported to show anxiety, as opposed to pleasure, in response to food intake (Kaye, Wierenga, Bailer, Simmons, & Bischoff-Grethe, 2013) and AMPH administration (Bailer et al., 2012). Individuals with acute AN have also been shown to exert more self-control, relative to healthy control subjects, in rejecting or delaying the receipt of reward (Decker, Figner, & Steinglass, 2015; Steinglass et al., 2012). Interestingly, individuals with AN who exercise excessively tend to be more anhedonic than non-exercisers (Davis & Woodside, 2002), making anhedonia particularly relevant in the ABA model. Anhedonia has not, however, been explicitly assessed in the context of ABA¹. Using a sucrose preference procedure as a measure of anhedonia, we examined whether ABA-susceptible rats displayed anhedonia prior to their experience with ABA.

Another behavioural measure of depression-like symptoms in rodents is the FST which was used and discussed in chapter 3. Prior to this dissertation, FST had not been assessed in the context of ABA. While ABA did not result in depression-like behaviours as measured by the

¹ Since the completion of the present experiment, Milton, Oldfield, and Foldi (2018) published a study in which they investigated anhedonia using a sucrose preference task in rats exposed to ABA. Their findings will be included in the general discussion (Chapter 7).

FST in the female rats in experiment 1.3, we did observe individual variability in response to FST at baseline (prior to ABA) in both male and female rats. Here, we assessed whether baseline depression-like behaviours in the FST could predict ABA susceptibility.

In addition to depressive symptoms, anxiety is frequently observed in AN. Individuals who develop anxiety disorders in childhood have been reported to be at increased risk of developing AN in adulthood (Kaye et al., 2004). Furthermore, anxiety symptoms have been found to contribute to higher physical activity in acute AN leading some researchers to suggest that exercise may have an anxiolytic function, especially during early stages of illness (Guarda et al., 2015; Holtkamp, Hebebrand, & Herpertz-Dahlmann, 2004). Anxiety also appears to play a role in ABA development in rodents. For instance, certain strains of mice that have been shown to be more susceptible to ABA also seem to exhibit higher anxiety (Gelegen et al., 2007). Anxiety in rodents has also been associated with higher running wheel activity (Wable et al., 2015). Studies aimed at examining the long-term effect of ABA in rodents show that rats exposed to ABA during adolescence display increased anxiety-like behaviours on the EPM in adulthood (Lee & Kinzig, 2017). To the best of our knowledge, however, there have been no studies examining whether baseline anxiety-like behaviours predict later ABA susceptibility in adult rats.

To summarize, the goal of experiment 2.1 was to determine whether baseline depression-like and anxiety-like behaviours could predict ABA susceptibility. We expected that ABA susceptible rats, compared to ABA resilient rats, would show more behaviours indicative of anhedonia and learned helplessness on the SPT and FST, respectively, and would display more anxiety-like behaviour in the EPM prior to the onset of ABA.

Method

Subjects

Female Sprague Dawley rats ($n = 12$; 125-150 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec) and housed in a colony room on a 12:12 hr reverse light/dark cycle. Upon arrival, rats were initially pair-housed in plastic shoebox cages and were then separated into individual shoebox cages on day 3. Rats underwent behavioural testing (EPM, FST, and SPT – described below) from day 7 to 13. On day 16, rats were transferred to

the laboratory where they were permanently housed in running wheel cages inside sound-attenuating boxes until the end of the experiment. With the exception of the restriction phase, rats had *ad libitum* access to both food and water throughout the experiment and body weight (g), food intake (g), and water intake (g) was monitored daily at ZT 11-12.

Apparatus

Running Wheel Cages. See “General Methodology” section.

Elevated Plus Maze. See “General Methodology” section.

Forced Swim Task. See “General Methodology” section.

Procedure

The detailed timeline is depicted in Figure 4.1.

Elevated Plus Maze. EPM testing occurred on day 7 during a 3-hour time window corresponding to ZT 15-18, where ZT 0 is lights on. See “General Methodology” section for more details.

Forced Swim Task. FST took place on days 9 and 10, 2-3 days after the EPM. The task was administered during the active phase (ZT 15-18) under regular light conditions. See “General Methodology” for the detailed procedure.

Sucrose Preference. Anhedonia-like behaviour was assessed using the SPT with a 5% sucrose solution. On day 9, rats were given *ad libitum* access to two water bottles filled with tap water in order for them to habituate to having two bottles to drink from. Given rats’ natural neophobia, rats were allowed to habituate to the sucrose solution on day 10 by filling up one bottle with regular tap water and the other with the sucrose solution – position of the sucrose bottle was counterbalanced. Finally, on day 11, rats were again given access to a tap water bottle and a sucrose solution bottle and the amount consumed (g) from each bottle over the 24-hour period was recorded. Sucrose preference was calculated as a ratio of total fluid consumed (e.g., sucrose water intake/total fluid intake).

Wheel Habituation Phase. On day 16, rats were transferred to the running wheel cages and had unrestricted access to the running wheels, food, and water for 18 days.

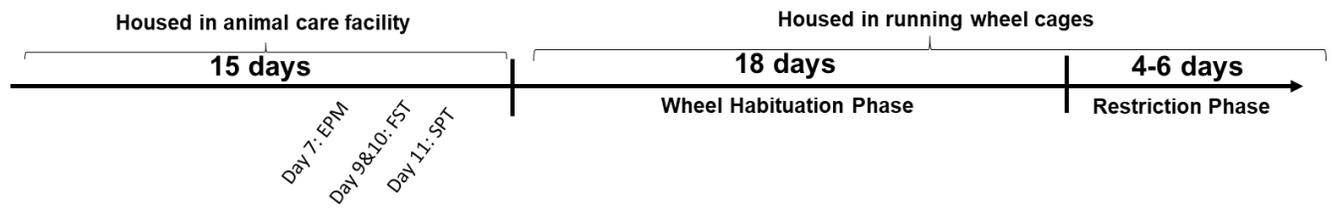


Figure 4.1. Timeline of experiment 2.1. EPM = Elevated Plus Maze; FST = Forced swim test; SPT = Sucrose preference test.

Restriction Phase. On day 33, the first day of the restriction phase, food was removed at ZT 13. On the following days, rats had access to food for 1 hr/day between ZT 12-13. The restriction phase was terminated when a rat reached starvation criterion (weight loss of 25% of initial body weight).

Statistical Analysis

A median split on percent of initial body weight after 4 days of ABA was used to retrospectively assign rats to the resilient or susceptible groups whereby rats with the lowest percent body weight (i.e., those that lost the most weight during ABA) were considered susceptible (DeCoster, Gallucci, & Iselin, 2011). These two groups were then used as categorical independent variables in a series of ANOVAs and *t*-tests. It should be noted that linear regression was also considered as a potential way of analysing the data (e.g., using continuous data from the EPM, FST, and SPT to predict percent of initial body weight after 4 days of ABA). We opted for the ANOVA and *t*-tests to maintain consistency with the statistical analyses used in this dissertation. Furthermore, with 6 rats per group, we did not have the power required for a regression analysis including so many predictors.

To characterize changes across the 18 days of the wheel habituation phase in resilient and susceptible rats, three separate two-way mixed ANOVAs were used for each of the following dependent variables: running wheel activity, body weight, and food intake. The between-subjects factor was *trait* (resilient vs. susceptible) and the within-subjects factor was *days* (18 days of the wheel habituation phase).

To characterize changes from the wheel habituation phase to the restriction phase in resilient and susceptible rats, three separate 2 x 2 mixed ANOVAs were used for the following dependent variables: running wheel activity, food intake, and body weight. The between-subjects factor was *trait* (resilient vs. susceptible) and the within-subjects factor was *phase* (habituation phase vs. restriction phase). The habituation phase represented the average across the last 3 days of habituation before the start of restriction phase for each of the dependent variable of interest. The restriction phase was represented by the average across the first 3 days of this restriction phase for each of the dependent variable of interest.

A series of one-tailed independent-samples *t*-tests were used to test for differences between resilient and susceptible rats on the EPM, FST, and SPT. Trait was used as the independent variable and dependent variables included proportion of time in open arms and closed arm entries for the EPM, latency to immobility, immobility time, and climbing time for the FST, and sucrose preference ratio for the SPT.

Results

Identification of a susceptibility trait

The restriction phase lasted a maximum of 6 days. There was considerable variability in survival days (i.e., number of restriction days required for rats to reach the starvation criterion of 75% of their initial body weight). While some rats reached starvation criterion within as little as 4 days, others maintained a body weight above the starvation criterion until the end of the restriction phase. In an effort to verify the veracity of the resilient-susceptible median split based on percent of initial body weight after 4 days of ABA, we examined the relationship between percent of initial body weight after 4 days of ABA and average wheel rotation in the 4 last days of wheel habituation, average wheel rotations during the first 4 days of ABA, percent change in wheel activity from wheel habituation to ABA (i.e., starvation-induced hyperactivity), and average food intake during the first 4 days of ABA (Figure 4.2). We found that a lower percent of initial body weight after 4 days of ABA was associated with higher running wheel activity during wheel habituation ($r = -.781, p = .001$; Figure 4.2A) and during ABA ($r = -.804, p = .001$; Figure 4.2B). Though not statistically significant, there was a trend for a negative correlation between percent of initial body weight after 4 days of ABA and percent change in wheel activity from wheel habituation to ABA meaning that rats with lower percent of initial body weight showed more starvation-induced hyperactivity ($r = -.445, p = .074$; Figure 4.2C). There was no correlation between percent of initial body weight after 4 days and food intake during ABA ($r = .157, p = .313$; Figure 4.2D).

Running Wheel Activity

Wheel habituation phase. As seen in Figure 4.3A, running wheel increased significantly across the 18 days of the wheel habituation phase and susceptible rats ran significantly more than

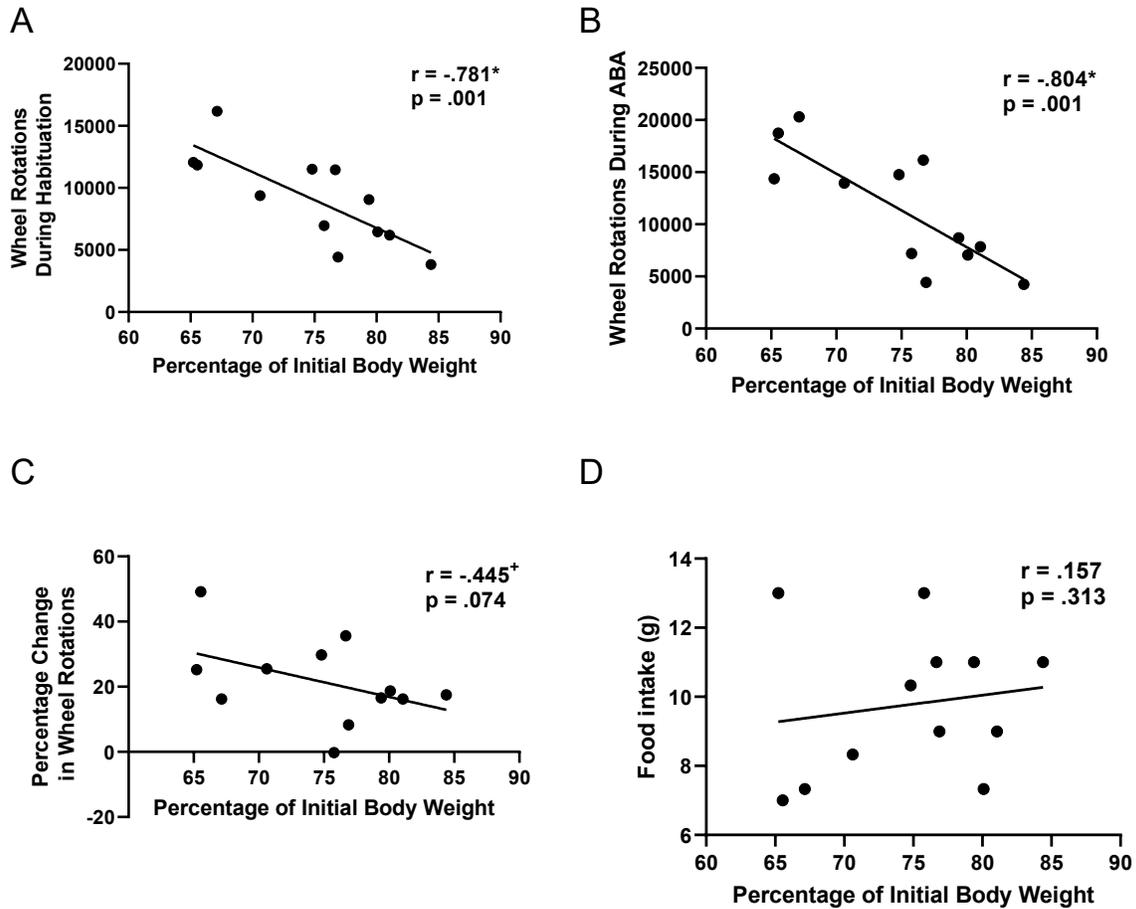


Figure 4.2. Relation between percentage of initial body weight in ABA and running wheel activity and food intake. Scatterplots depicting the correlations between percentage of initial body weight after 4 days of ABA and (A) average wheel rotations during the last 4 days of the wheel habituation phase, (B) average wheel rotations during the first 4 days of ABA, (C) percent change in wheel rotations from the wheel habituation phase to the ABA phase, and (D) average food intake during the first 4 days of ABA. $*p < .05$, $+p < .10$.

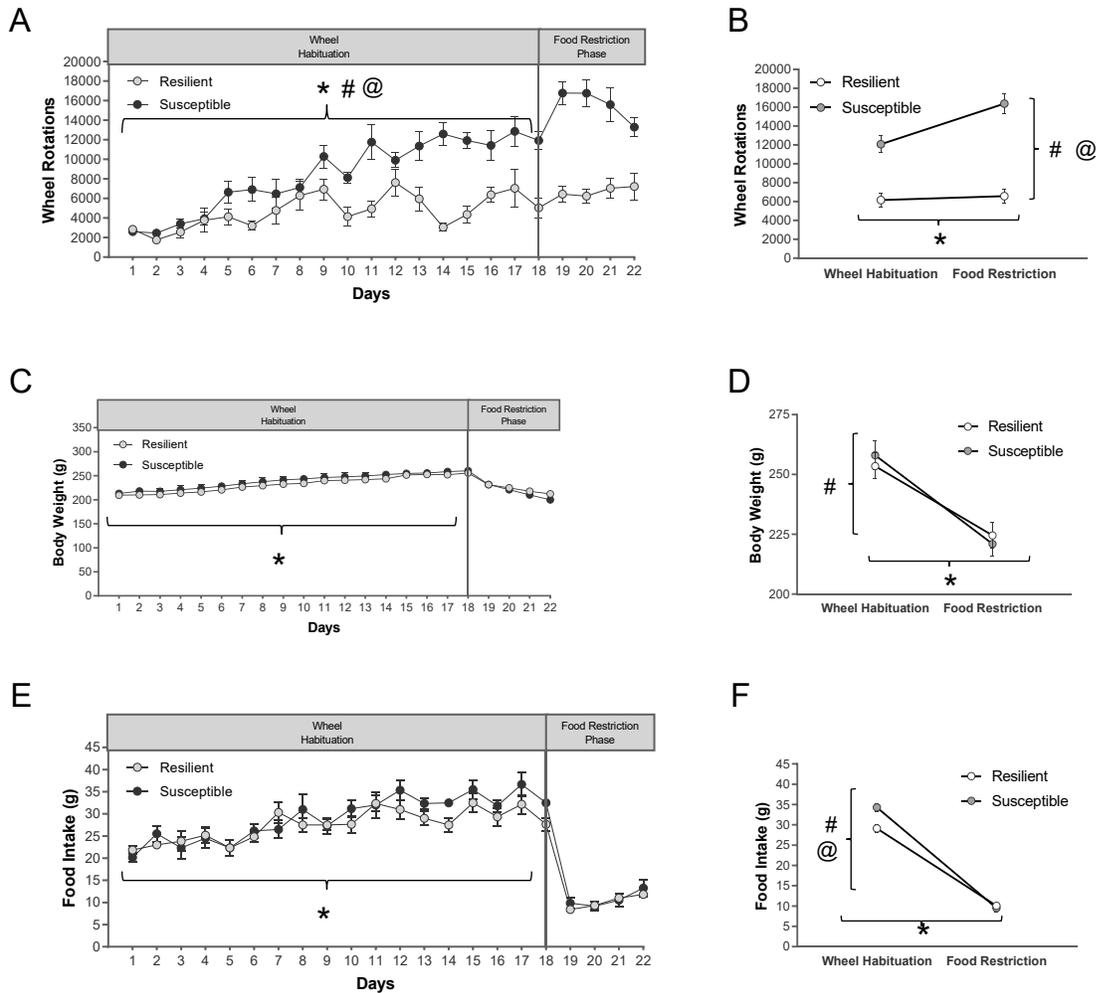


Figure 4.3. Running wheel activity, body weight, and food intake before and during ABA in resilient ($n = 6$) and susceptible ($n = 6$) rats. (A) Mean daily wheel rotations across experimental days. * $p < .001$, main effect of days; # $p < .001$, main effect of trait; @ $p < .001$, days \times trait interaction. (B) Mean daily wheel rotations across the last 3 days of habituation compared to the first 3 days of the restriction phase. * $p < .001$, main effect of phase; # $p < .001$, main effect of trait; @ $p < .001$, phase \times trait interaction. (C) Mean daily body weight across experimental days. * $p < .001$, main effect of days. (D) Mean daily body weight across the last 3 days of habituation compared to the first 3 days of the restriction phase. * $p < .001$, main effect of phase; @ $p = .003$, phase \times trait interaction. (E) Mean daily food intake across experimental days. * $p < .001$, main effect of days. (F) Mean daily food intake across the last 3 days of habituation compared to the first 3 days of the restriction phase. * $p < .001$, main effect of phase; # $p = .033$, main effect of trait; @ $p = .009$, phase \times trait interaction.

resilient rats. Importantly, susceptible rats, compared to resilient rats, increased their running wheel activity more rapidly across habituation days (*days*: $F(17,170) = 11.70, p < .001, \eta_p^2 = .54$; *trait*: $F(1,10) = 38.21, p < .001, \eta_p^2 = .79$; *days x trait*: $F(17, 170) = 4.04, p < .001, \eta_p^2 = 0.29$).

Restriction phase. Average wheel rotations across the first 3 days of the restriction phase was significantly higher than the average wheel rotations across the last 3 days of the wheel habituation phase (*phase*: $F(1,10) = 46.25, p < .001, \eta_p^2 = .82$) and, consistent with the above results, susceptible rats ran significantly more than resilient rats (*trait*: $F(1,10) = 43.18, p < .001, \eta_p^2 = .81$; Figure 4.3B). Importantly, the onset of food restriction resulted in hyperactivity in susceptible rats only (*phase x trait*: $F(1,10) = 31.34, p < .001, \eta_p^2 = .79$).

Body Weight

Wheel habituation phase. Rats' body weight (g) during running wheel habituation days is depicted in Figure 4.3C. Body weight gradually increased across the 18 habituation days (*days*: $F(17,170) = 169.64, p < .001, \eta_p^2 = .94$). There was no statistically significant main effect of *trait* or *days x trait* interaction (*trait*: $F(1,10) = 0.70, p = .423, \eta_p^2 = .07$; *days x trait*: $F(17,170) = 0.67, p = .831, \eta_p^2 = .06$).

Restriction phase. As expected, rats' body weight during the restriction phase (averaged across the first 3 days of the restriction phase) was significantly lower than that of the habituation phase (averaged across the last 3 days of habituation). While there was no statistically significant main effect of *trait*, susceptible rats weighed more than resilient rats during habituation, and the reverse was true for the restriction phase with susceptible rats weighing less than the resilient rats (Figure 4.3D; *phase*: $F(1, 10) = 967.11, p < .001, \eta_p^2 = .99$; *trait*: $F(1, 10) = 0.04, p < .001, \eta_p^2 = 1.00$; *phase x trait*: $F(1, 10) = 14.78, p = .003, \eta_p^2 = .60$).

Food Intake

Wheel habituation phase. Daily food intake gradually increased across the 18 wheel habituation days (Figure 4.3E; *days*: $F(17,170) = 13.91, p < .001, \eta_p^2 = .58$). There was no difference in food intake between the resilient and susceptible rats (*trait*: $F(1,10) = 4.02, p = .073, \eta_p^2 = .29$; *days x trait*: $F(17,170) = 1.21, p = .262, \eta_p^2 = .11$).

Restriction phase. As expected, food intake averaged across the first 3 days of the restriction phase was significantly lower than the average food intake across the last 3 days of the habituation phase (*phase*: $F(1,10) = 613.98, p < .001, \eta_p^2 = .98$). Overall, susceptible rats ate significantly more than resilient rats (*trait*: $F(1,10) = 6.10, p = .033, \eta_p^2 = .38$). However, susceptible rats ate more than resilient rats during the habituation phase but not during the restriction phase (*phase x trait*: $F(1,10) = 10.46, p = .009, \eta_p^2 = .51$; Figure 4.3F).

Elevated Plus Maze

There were no statistically significant differences between resilient and susceptible rats in proportion of time spent in the open arms ($t(10) = 0.03, p = .511, d = 0.02$; Figure 4.4A) or number of closed arm entries ($t(10) = 0.11, p = .919, d = 0.06$; Figure 4.4B)

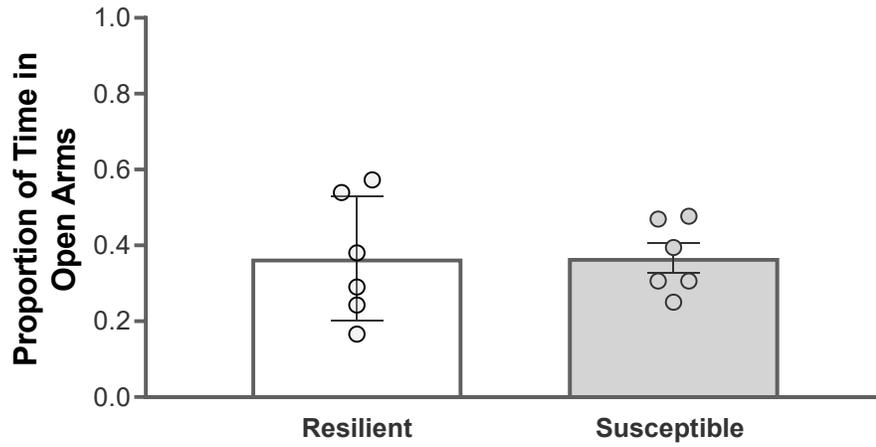
Forced Swim Task

Susceptible rats, relative to resilient rats, had a significantly longer latency to immobility ($t(10) = 2.72, p = .022, d = 1.57$; Figure 4.5A), spent less time immobile ($t(10) = 2.29, p = .045, d = 1.32$; Figure 4.5B), and spent more time climbing ($t(10) = 2.19, p = .053, d = 1.27$, Figure 4.5C)

Sucrose Preference Test

There was no statistically significant difference in sucrose preference ratio between resilient and susceptible rats ($t(10) = 1.36, p = .205, d = 0.78$; Figure 4.6).

A



B

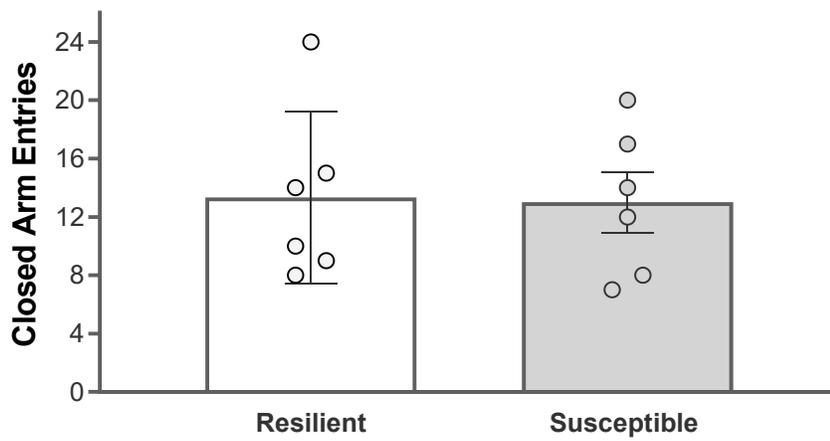


Figure 4.4. Behaviour in the elevated plus maze in resilient ($n = 6$) and susceptible ($n = 6$) rats prior to experiencing ABA. (A) Mean proportion of time in the open arms. (B) Mean closed arm entries.

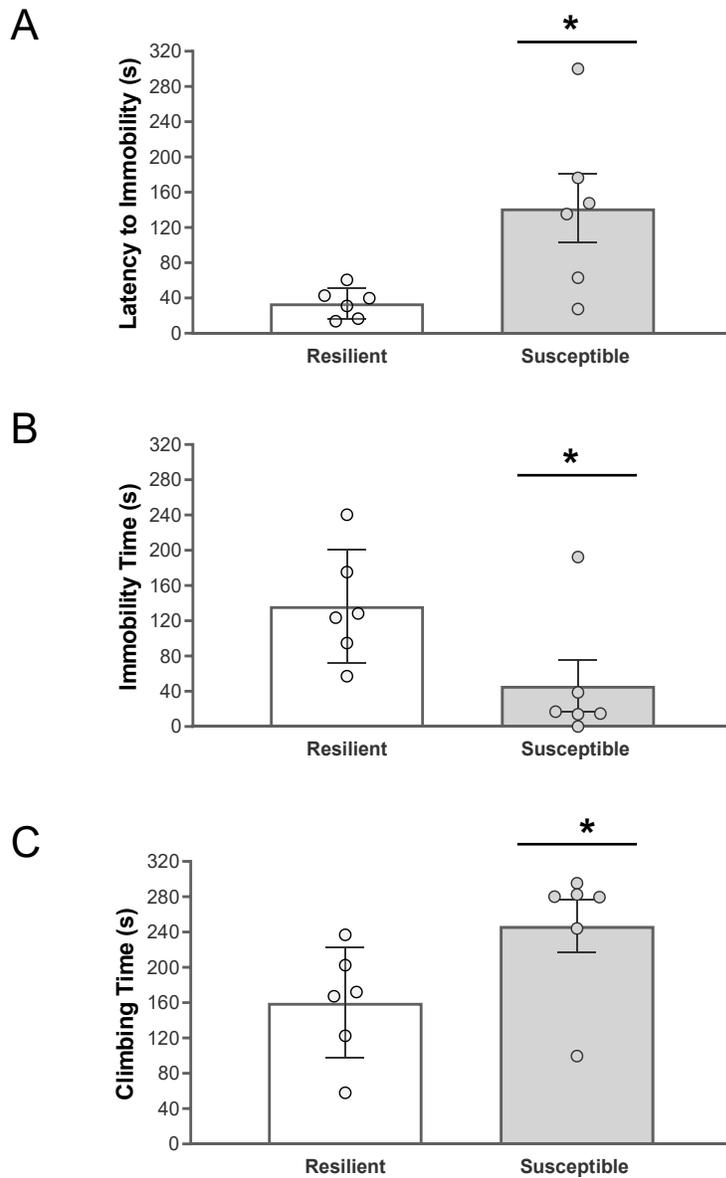


Figure 4.5. Behaviour in the forced swim test in resilient ($n = 6$) and susceptible ($n = 6$) rats prior to experiencing ABA. (A) Mean latency to immobility (in seconds). $*p = .022$, compared to resilient rats. (B) Mean immobility time (in seconds). $*p = .045$, compared to resilient rats. (C) Mean time spent climbing (in seconds). $*p = .053$, compared to resilient rats.

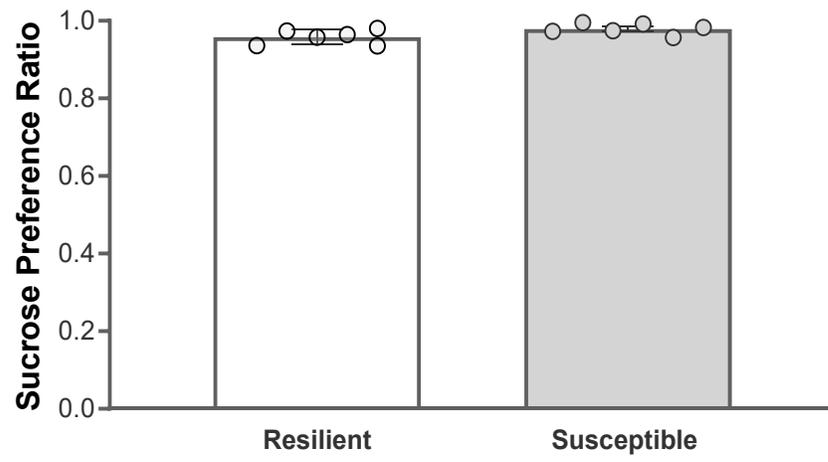


Figure 4.6. Sucrose preference ratio during a two-bottled sucrose preference test in resilient ($n = 6$) and susceptible ($n = 6$) rats prior to experiencing ABA.

Discussion

This experiment was the first of the present dissertation designed to investigate baseline differences between ABA-resilient and ABA-susceptible rats. Prior to the onset of ABA, rats were assessed for anxiety-like behaviours on the EPM and depression-like behaviours on the FST and SPT. It was hypothesized that susceptible rats, relative to resilient rats, would display more anxiety- and depression-like behaviours.

Identification of a susceptible trait

A median split based on percent of initial body weight on the 4th day of ABA was used to identify resilient and susceptible rats. We found this to be a reliable way of identifying susceptibility as rats who were labeled as susceptible based on this criterion were also the rats who showed the largest food restriction-induced hyperactivity (increase in running wheel activity from habituation to restriction). The resilience and susceptibility dichotomy could be further strengthened by using subjects at both extremes and omitting the rats that fall closer to the median. This approach, however, does require a larger sample size than the one used in the present study. We also found that rats identified as susceptible had higher baseline running wheel activity than resilient rats prior to the onset of the restriction phase. This finding suggests that baseline running wheel activity can be used as a predictor of ABA susceptibility and is consistent with reports from other researchers (Barbarich-Marsteller et al., 2013; Milton et al., 2018; Perez-Leighton et al., 2014; Pjetri et al., 2012).

Anxiety-like behaviours on the EPM did not predict ABA susceptibility

Our results suggest that resilient and susceptible rats do not differ on the EPM prior to the onset of ABA. Increased anxiety-like behaviours have been shown to be increased during ABA and after recovery from ABA, but not necessarily before ABA. In female adolescent mice undergoing ABA, hyperactivity was associated with more anxiety-like behaviours in the EPM, leading the authors to suggest that hyperactivity during ABA may serve as an indirect measure of anxiety (Wable et al., 2015). As is the case in humans, the anxiety-like behaviours during ABA do not appear to merely be a consequence of acute food restriction and weight loss as rats having experienced two bouts of ABA during adolescence display more anxiety-like behaviour in the EPM during adulthood (Lee & Kinzig, 2017). The present experiment was the first to investigate

whether there was an association between anxiety-like behaviours on the EPM before undergoing ABA. While Wable et al. (2015) used the EPM during ABA, they did use an open field test prior to ABA and found that there was no correlation between time spent in the center in the open field test (less time in center indicating higher anxiety) and later hyperactivity during ABA. Our results are therefore consistent with those of Wable et al. (2015) and suggest that baseline anxiety, as measured by the EPM, does not predict ABA susceptibility in the ABA model.

Running wheel activity was not related to general locomotor activity in the EPM

Total arm entries in the EPM are considered a measure of general locomotor activity (Lister, 1987; Walf & Frye, 2007). Given that baseline running wheel activity predicted ABA-susceptibility in the present experiment, it would be tempting to assume that susceptible rats are generally more active than resilient rats. This, however, was not the case as no differences in total arm entries were observed between resilient and susceptible rats. Wable et al. (2015) also found this to be true in adolescent mice whereby wheel activity was not correlated with total arm entries. These findings, however, are in conflict with those of Perez-Leighton et al. (2014) who found that baseline spontaneous physical activity before ABA (measured by infrared activity sensors placed around a cage) predicted baseline running wheel activity and ABA susceptibility. The relationship, however, was complex in that either very high or very low spontaneous physical activity increased the probability of ABA susceptibility and that the effect was larger in male rats compared to female rats. It should also be noted that the total arm entries in the present experiment represent locomotor activity in a novel environment while the spontaneous physical activity measured by Perez-Leighton et al. (2014) was measured in an environment to which the rats had acclimated. It is thus possible that the novelty of the environment masked individual variability in locomotor activity. It is also possible that running wheel activity is a specific type of activity distinct from general exploratory behaviour. Indeed, it has been suggested that running wheel activity should be considered a behaviour in and of itself reflecting several underlying behavioural processes in addition to an animal's general spontaneous activity (see review by Novak, Burghardt, & Levine, 2012). More research is required to clarify the relation between spontaneous locomotor activity and running wheel activity and their relation to ABA susceptibility.

ABA susceptibility was associated with longer latency to immobility and less time spent immobile in the FST prior to ABA

This finding did not support our hypothesis and was in fact in the opposite direction. Based on the classical interpretation of behaviour in the FST, our results would suggest that ABA-susceptible rats, compared to resilient rats, showed less depression-like behaviours or, more specifically, less behavioural despair, at baseline before ABA. This contradicts the human literature in which depression has been shown to be highly comorbid with AN though the chronology of depressive symptoms in AN is less clear (Fornari et al., 1992; Godart et al., 2015). The interpretation of our FST results are discussed in more detail in the general discussion (Chapter 7).

There was no difference in sucrose preference between resilient and susceptible rats

Anhedonia is a hallmark symptom of AN and has been proposed as one of the mechanisms contributing to disease maintenance. Using the SPT to assess anhedonia, we hypothesized that susceptible rats would show anhedonia-like behavior (i.e., lower sucrose preference ratio) compared to resilient rats prior to the start of ABA. Our results did not support this hypothesis as no differences between resilient and susceptible rats were observed. At the time of the present experiment, this was the first attempt to assess anhedonia-like behaviours in the context of ABA. One study has since been published and reports findings that are consistent with ours (Milton et al., 2018). Our SPT results, along with those of Milton et al. (2018) are discussed in further detail in the general discussion (Chapter 7). Future studies investigating the role of anhedonia in ABA would benefit from using tasks that assess motivation to work for a reward in addition to the SPT. Such tasks include, but are not limited to, intracranial self-stimulation, drug self-administration, effort-related choice behaviours, and probabilistic reward learning tasks, and are discussed in a review by Scheggi, De Montis, & Gambarana (2018).

EXPERIMENT 2.2. AMPHETAMINE-INDUCED LOCOMOTOR ACTIVITY BEFORE AND AFTER ABA

Genetic, pharmacological, and physiological data have pointed towards altered DA functioning in AN and ABA. The role of DA in feeding behaviour has been studied extensively with the nigrostriatal DA pathway being associated with the sensory-motor aspects of feeding (Smith & Schneider, 1988) while the mesolimbic DA system has generally been associated with food motivation and reward (Palmiter, 2007; Salamone & Correa, 2002; Smith & Schneider, 1988). Beyond its involvement in feeding and appetite, DA may also play a role in many of the other symptoms of AN such as hyperactivity (Joyce & Koob, 1981; Koob & Swerdlow, 1988), body image distortions and stereotyped behaviours (Ellinwood, Sudilovsky, & Nelson, 1973), and disruptions in cognitive functions such as decision-making (Rogers et al., 1999), executive control (Roesch-Ely et al., 2005), and cognitive flexibility (Roberts et al., 1994).

While preclinical and clinical studies of AN have provided evidence for a disruption in DA functioning in AN, the exact nature of this disruption is complex. Evidence that the DA system is involved in AN includes reduced cerebrospinal (CSF) levels of DA metabolites in both ill individuals and those recovered from AN (Kaye, Ebert, Raleigh, & Lake, 1984; Kaye, Frank, & McConaha, 1999), functional DA D2 receptor gene polymorphisms in individuals with AN (Bergen et al., 2005), and impaired visual discrimination learning which is thought to reflect DA-signalling function, in individuals with AN (Lawrence et al., 2003). Evidence from neuroimaging studies also supports a role for altered central reward processing in the development and maintenance of AN. For example, individuals with AN have been shown to have reduced neural activity in the striatum in response to sucrose (Wagner et al., 2008). Additionally, a PET study found that individuals who recovered from AN had increased D2/D3 receptor binding in the ventral striatum, a region known to modulate responses to reward stimuli (Frank et al., 2005). This could indicate increased D2/D3 densities, decreased extracellular DA, or both, in recovered individuals. Broft et al. (2015), however, found no changes in D2 availability in acute AN. The clinical literature summarized here provides evidence for altered DA in AN though the putative direction of this alteration remains unclear.

Several lines of preclinical research have also implicated DA in AN-related symptoms in rodents. For instance, absence of tyrosine hydroxylase in DA neurons results in hypophagia in

mice (Zhou & Palmiter, 1995). Furthermore, selective depletion of DA neurons with 6-hydroxydopamine results in hypoactivity and hypophagia (Zigmond & Stricker, 1972). In ABA, systemic treatment with DA antagonists has been shown to promote body weight maintenance and survival by increasing food intake or suppressing hyperactivity (Hillebrand, Van Elburg, et al., 2005; Klenotich et al., 2015; Verhagen, Luijendijk, Korte-Bouws, et al., 2009). Selective DA D1 receptor agonists have been shown to reduce food intake in free-feeding rats (Cooper, Al-Naser, & Clifton, 2006). In rats exposed to ABA, tetrahydrocannabinol, which increases DA levels in the striatum (Malone & Taylor, 1999), has been shown to increase highly palatable food consumption leading to attenuation of weight loss (Verty et al., 2011). These seemingly contradictory findings highlight the complexity of global versus pathway-specific DA effects in ABA.

Given the above reports of DA disruption in both AN and ABA, we aimed to examine whether disrupted DA signalling played a role in ABA susceptibility. At the time of the present study, the mesolimbic DA system had not been specifically investigated in rodents exposed to ABA². We aimed to investigate whether baseline differences in mesolimbic DA functioning existed between ABA resilient and susceptible rats prior to being exposed to ABA as well as following an episode of ABA. To do so, locomotor activity was assessed following several AMPH challenges. AMPH is a stimulant drug that increases extracellular levels of DA in the striatal regions via release and reverse transport, resulting in increased locomotor activity (Koob, 1992). Hyperactivity responses to AMPH in rodents is frequently used as an index of underlying mesolimbic DA activity. Based on the notion that anhedonia and altered reward processing, via the mesolimbic DA pathway, may underlie AN and AN-like behaviours in ABA, it was hypothesized that ABA susceptible rats, compared to ABA resilient rats, would show an attenuated locomotor response to AMPH challenges both before and after ABA.

Method

Subjects

² Since the completion of this experiment, Foldi, Milton, and Oldfield (2017) published a study examining the effects of activating of the VTA-NAc pathway using DREADD technology on ABA development. Their findings will be discussed relative to our findings in the present experiment's discussion.

Twenty-four female Sprague Dawley rats (125-150 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec). Twelve rats were used in experiment 2.2.1 and the remaining 12 were used in experiment 2.2.2. As in previous experiments, rats were kept on a 12:12 hr reverse light/dark cycle and their body weight, food intake, and water intake were monitored daily at ZT 11-12 throughout the experiments. Upon arrival, rats were individually housed in a colony room and allowed to acclimate for 7 days before being relocated into running wheel cages. With the exception of the restriction phase, rats had *ad libitum* access to both food and water throughout the experiment.

Apparatus

Running wheel cages. See “General Methodology” section.

Locomotor activity boxes. Locomotor activity was quantified with an infrared activity-monitoring apparatus for rats (Truscan, Coulbourn Instruments, Whitehall, PA, USA). The apparatus were clear Perspex test chambers measuring 39 cm X 42 cm X 50 cm with 16 x 16 light-emitting diode photodetector pairs located along the base and spaced every 2.5 cm. Data were captured using Truscan2 software in total distance travelled in centimeters. All rats were tested throughout the experiment in the same respective activity chamber at the same time of day.

Drugs

D-Amphetamine sulfate (AMPH, Sigma-Aldrich) was dissolved in 0.9% saline to concentrations of 0.50, 0.75, and 1.00 mg/ml.

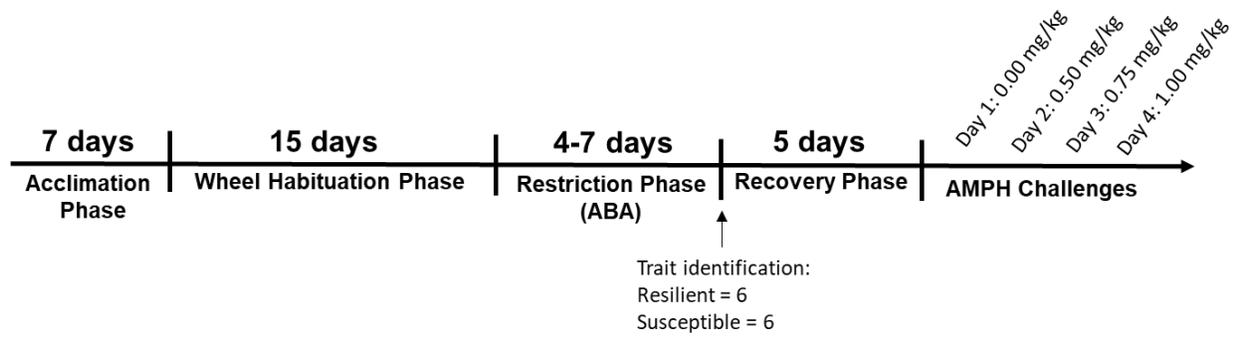
Procedure

Experiment 2.2.1: AMPH-induced locomotor after experiencing ABA. The timeline for experiment 2.2.1 can be seen in Figure 4.7A. Following a 7-day acclimation period in the animal care facility, all rats underwent the following procedure.

Running wheel habituation phase. On day 8, rats were transferred to the running wheel cages and had unrestricted access to the running wheels, food, and water for 15 days.

Restriction phase. On day 23, the first day of the restriction phase, food was removed at ZT 13. On the following days, rats had access to food for 1 hr/day between ZT 12-13. The

A



B

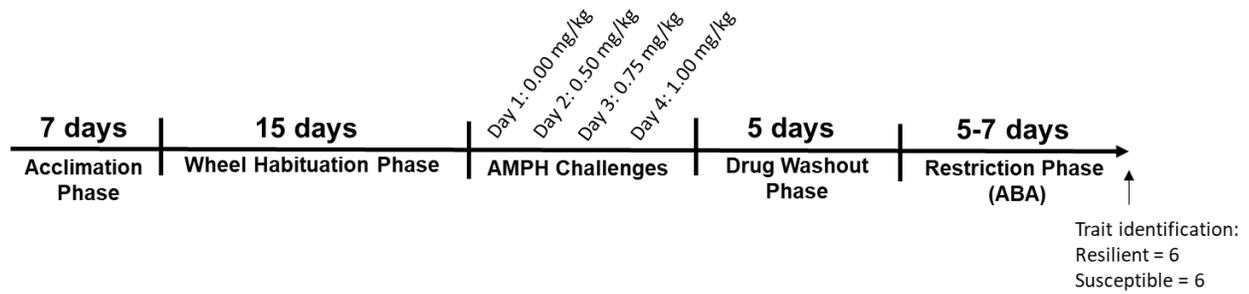


Figure 4.7. Timeline for experiment 2.2.1 (A) and 2.2.2 (B).

restriction phase was terminated when a rat reached survival criteria (weight loss of 25% of initial body weight).

Recovery phase. Once rats had lost 25% of their initial body weight, they were allowed to recover for 5 days. During this recovery period, rats had *ad libitum* access to food and water with continued access to the running wheels.

AMPH-induced locomotor activity. Locomotor activity testing occurred over a 4-day period. On day 1, rats were placed in the box for 30 min prior to injection. Following this habituation period, all rats received an injection of 0.9% saline (0.00 mg/kg, i.p.) after which they were left in the activity box for an additional 60 min. On days 2, 3, and 4, rats underwent the same procedure but received an i.p. injection of 0.50 mg/kg, 0.75 mg/kg, and 1.00 mg/kg AMPH, respectively. Each rat was tested in the same activity box and at the same time of day across the 4 days.

Experiment 2.2.2: AMPH-induced locomotor activity prior to experiencing ABA. The timeline for experiment 2.2.2 can be seen in Figure 4.7B. Following a 7-day acclimation period in the animal care facility, all rats underwent the following procedure.

Running wheel habituation phase. On day 8, rats were transferred to the running wheel cages and had unrestricted access to the running wheels, food, and water for 15 days.

AMPH-induced locomotor activity. Locomotor activity testing occurred over a 4-day period as described above in experiment 2.2.1.

Drug washout phase. AMPH-induced locomotor activity testing was followed by a 5-day washout period during which rats remained in their running wheel cages and continued to have unrestricted access to food, water, and the running wheel.

Restriction phase. Following 5 days of drug washout, the food restriction phase began as described in experiment 2.2.1. Rats exited the experiment when they lost 25% of their initial body weight.

Statistical Analysis

A median split based on days to starvation criterion (25% weight loss) was used to divide rats into a resilient group and susceptible group. To examine the development of ABA, a series

of mixed 2 x 2 ANOVAs were used for each dependent variable: food intake, body weight, and running wheel activity. *Trait* was the between-subject factor and consisted of resilient or susceptible rats. *Phase* was used as the within-subject factor and consisted of the average food intake, body weight, or running wheel activity across the last 4 days (exp. 2.2.1) or 5 days (exp. 2.2.2) of the running wheel habituation phase versus the first 4 days (exp. 2.2.1) or 5 days (exp. 2.2.2) of the restriction phase. A series of mixed ANOVAs were used to analyze total distance travelled (cm) following saline and AMPH challenges. *Trait* was used as the between-subject factor and consisted of resilient versus susceptible rats. *Time* was used as the within-subject factor and consisted of 12 5-min time bins (exp. 2.2.1) and 18 5-min time bins (exp. 2.2.2) representing the time following injection.

Results

Experiment 2.2.1: AMPH-induced locomotor after experiencing ABA

Data integrity. One rat from the susceptible group escaped the locomotor activity box during the 0.75 mg/kg and 1.00 mg/kg AMPH challenges resulting in missing data. For this reason, this rat was excluded from locomotor activity analyses for these two days.

Development of ABA. Days required to reach the starvation criterion varied from 4 to 7 days. Running wheel activity, food intake, and body weight during the wheel habituation and restriction phases are depicted in Figure 4.8 with ANOVA results. In summary, during the wheel running habituation phase, there were no differences between resilient and susceptible rats in food intake and body weight. Running wheel activity during both phases was higher in susceptible rats compared to resilient rats. During the restriction phase, susceptible rats did not eat more or less than the resilient rats. As expected, however, they showed increased hyperactivity compared to the resilient rats, resulting in accelerated weight loss. See supplementary materials for descriptive statistics and ANOVA tables.

AMPH-induced locomotor activity. Following the saline injection, ambulatory locomotion significantly decreased and eventually stabilized (*time*: $F(11,110) = 8.96$, $p < .001$, $\eta_p^2 = 0.47$), but there was no statistically significant difference between resilient and susceptible rats (*trait*: $F(1, 10) = 0.40$, $p = .397$, $\eta_p^2 = 0.07$), and no statistically significant interaction (*time x trait*: $F(11, 110) = 0.88$, $p = .561$, $\eta_p^2 = 0.08$; Figure 4.9A).

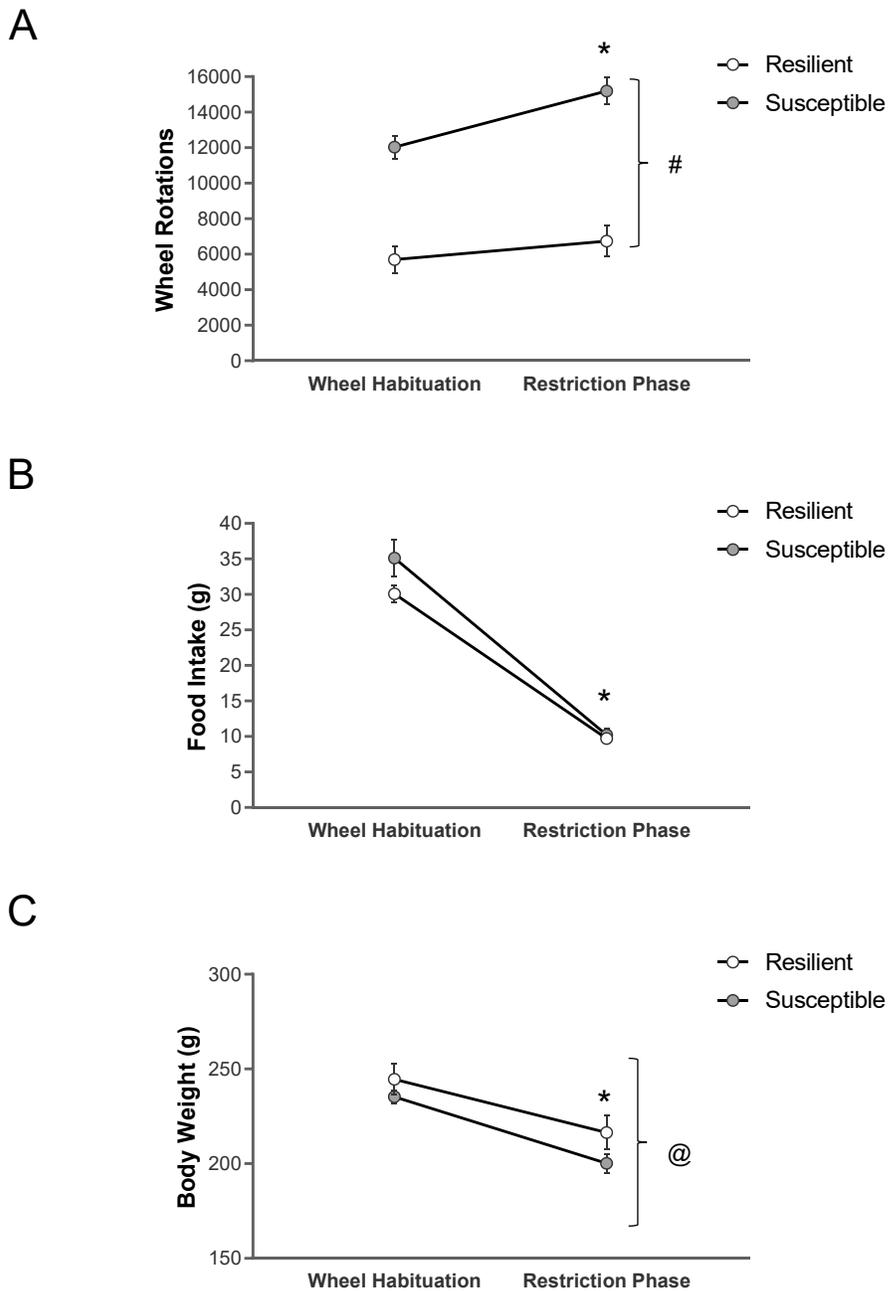


Figure 4.8. The effect of ABA on running wheel activity, food intake, and body weight (mean and SEM) averaged across the last 4 days of the habituation phase and the first 4 days of the restriction phase in resilient rats ($n = 6$) and susceptible rats ($n = 6$). (A) Running wheel activity during the habituation phase and the restriction phase. * $p < .001$, main effect of *phase*; # $p < .001$, main effect of *trait*. (B) Food intake during the habituation phase and the restriction phase. * $p < .001$, main effect of *phase*. (C) Body weight during the habituation phase and the restriction phase. * $p < .001$, main effect of *phase*. @ $p = .036$, *phase x trait* interaction.

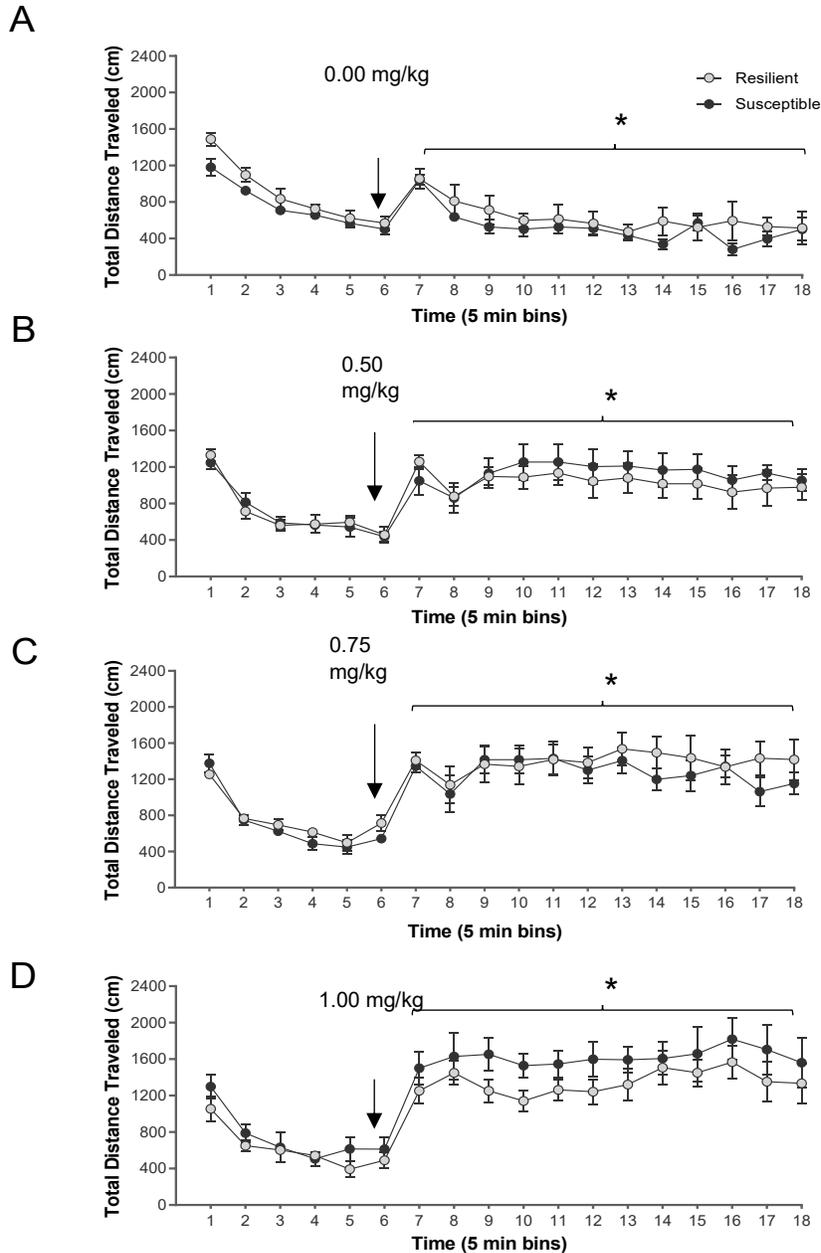


Figure 4.9. Locomotor activity following AMPH challenges in rats with a previous history of ABA. (A) Locomotor activity before and after a saline injection in resilient ($n = 6$) and susceptible ($n = 6$) rats. * $p < .001$, main effect of *time*. (B) Locomotor activity before and after a 0.50 mg/kg AMPH injection in resilient ($n = 6$) and susceptible ($n = 6$) rats. * $p = .002$, main effect of *time*. (C) Locomotor activity before and after a 0.75 mg/kg AMPH injection in resilient ($n = 6$) and susceptible ($n = 5$) rats. * $p = .005$, main effect of *time*. (D) Locomotor activity before and after a 1.00 mg/kg AMPH injection in resilient ($n = 6$) and susceptible ($n = 5$) rats. * $p = .010$, main effect of *time*.

The 0.50 mg/kg AMPH challenge resulted in a statistically significant increase in locomotor activity over time (*time*: $F(11, 110) = 2.93, p = .002, \eta_p^2 = 0.23$). There was no statistically significant difference between resilient and susceptible rats (*trait*: $F(1,10) = 0.20, p = .664, \eta_p^2 = 0.02$), and no statistically significant interaction (*time x trait*: $F(11, 110) = 1.07, p = .395, \eta_p^2 = 0.10$; Figure 4.9B).

Similarly, the 0.75 mg/kg AMPH challenge also resulted in a statistically significant increase in locomotor activity over time (*time*: $F(11, 99) = 2.63, p = .005, \eta_p^2 = 0.23$) with no statistically main effect of trait (*trait*: $F(1, 9) = 0.27, p = .619, \eta_p^2 = 0.03$) or interaction, (*time x trait*: $F(11, 99) = 1.40, p = .187, \eta_p^2 = 0.13$; Figure 4.9C).

The higher 1.00 mg/kg AMPH challenge also yielded similar results with a statistically significant increase in locomotor activity over time (*time*: $F(11, 99) = 2.44, p = .010, \eta_p^2 = 0.21$), but no statistically main effect of trait (*trait*: $F(1,9) = 1.18, p = .307, \eta_p^2 = 0.16$) or interaction (*time x trait*: $F(11, 99) = 0.95, p = .501, \eta_p^2 = 0.10$; Figure 4.9D).

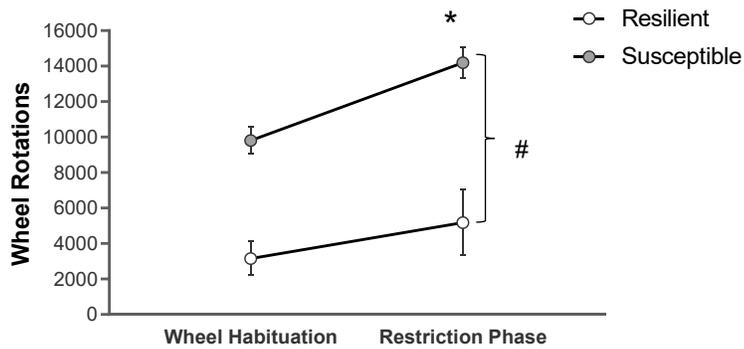
Experiment 2.2.2: AMPH-induced locomotor activity prior to experiencing ABA

Data integrity. Two rats (1 resilient and 1 susceptible) escaped the locomotor activity box during the saline challenge resulting in missing data. For this reason, these rats were excluded from locomotor activity analyses for the saline day.

Development of ABA. Days required to reach the starvation criterion varied from 5 to 7 days. Running wheel activity, food intake, and body weight during the wheel habituation and restriction phases are depicted in Figure 4.10 with ANOVA results. In summary during the wheel running habituation phase, there were no differences between resilient and susceptible rats in food intake and body weight. Running wheel activity during the wheel habituation phase was higher in susceptible rats compared to resilient rats. During the restriction phase, susceptible rats did not eat more or less than the resilient rats. As expected, however, they showed increased hyperactivity compared to the resilient rats, resulting in accelerated weight loss. See supplementary materials for descriptive statistics and ANOVA tables.

AMPH-induced locomotor activity. Following the saline injection, ambulatory locomotion significantly decreased and stabilized over time (*time*: $F(17, 136) = 4.18, p < .001$,

A



B



C

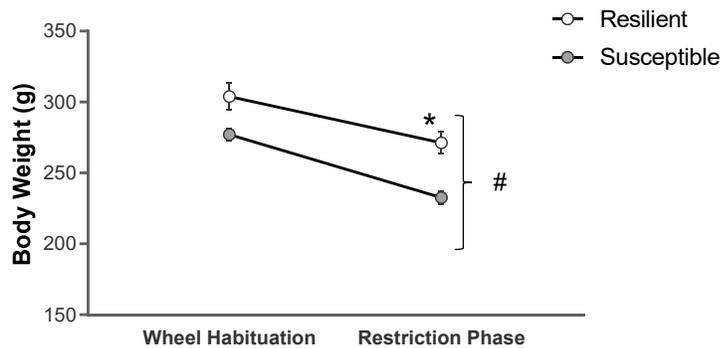


Figure 4.10. The effect of ABA on running wheel activity, food intake, and body weight (mean and SEM) averaged across the last 5 days of the habituation phase and the first 5 days of the restriction phase in resilient rats ($n = 6$) and susceptible rats ($n = 6$). (A) Running wheel activity during the habituation phase and the restriction phase. * $p = .001$, main effect of *phase*; # $p < .001$, main effect of *trait*. (B) Food intake during the habituation phase and the restriction phase. * $p < .001$, main effect of *phase*. (C) Body weight during the habituation phase and the restriction phase. * $p < .001$, main effect of *phase*. # $p = .001$, main effect of *trait*.

$\eta_p^2 = 0.34$), but there was no statistically significant difference between resilient and susceptible rats (*trait*: $F(1, 8) = 2.27, p = .171, \eta_p^2 = 0.22$) and no statistically significant interaction (*time x trait*: $F(17, 136) = 0.80, p = .689, \eta_p^2 = 0.09$; Figure 4.11A).

The 0.50 mg/kg AMPH challenge resulted in an increase in locomotor activity that gradually decreased across time (*time*: $F(17, 170) = 1.80, p = .032, \eta_p^2 = 0.15$). There was no statistically significant difference between resilient and susceptible rats (*trait*: $F(1,10) = 1.36, p = .271, \eta_p^2 = 0.12$), and no statistically significant interaction (*time x trait*: $F(17, 170) = 0.75, p = .749, \eta_p^2 = 0.07$; Figure 4.11B).

Similarly, the 0.75 mg/kg AMPH challenge also resulted in an increase in locomotor activity that decreased over time (*time*: $F(17, 170) = 3.67, p < .001, \eta_p^2 = 0.27$) with no statistically main effect of trait (*trait*: $F(1,10) = 0.34, p = .571, \eta_p^2 = 0.03$) or interaction (*time x trait*: $F(17, 170) = 0.54, p = .931, \eta_p^2 = 0.05$; Figure 4.11C).

The higher 1.00 mg/kg AMPH challenge also yielded similar results with a statistically significant effect of time (*time*: $F(17, 170) = 3.30, p < .001, \eta_p^2 = 0.25$), but no statistically main effect of trait (*trait*: $F(1,10) = 0.39, p = .545, \eta_p^2 = 0.04$) or interaction (*time x trait*: $F(17, 170) = 0.44, p = .972, \eta_p^2 = 0.04$; Figure 4.11D).

Discussion

Anhedonia and disruptions in reward-processing have been shown to be involved in AN and to be mediated by the mesolimbic DA system. At the time of the present experiment, however, mesolimbic DA functioning had not been examined in ABA. Using AMPH-induced hyperactivity as an index of increased mesolimbic DA, it was hypothesized that ABA susceptible rats would show suppressed AMPH-induced hyperactivity compared to ABA resilient rats both before and after ABA, reflecting mesolimbic DA hypoactivity. Our results did not support this hypothesis. Instead, we found that there were no differences between resilient and susceptible rats in AMPH-induced locomotion before experiencing ABA (exp. 2.2.1) or after ABA (exp 2.2.2). Based on these findings, we can conclude that baseline mesolimbic DA functioning before ABA does not predict ABA susceptibility. This finding is consistent with the results from the SPT in experiment 2.1 which suggested that susceptible rats did not show more anhedonia-like behaviours, mediated by mesolimbic DA, at baseline compared to resilient rats. It can also be concluded that susceptible rats do not display more long-term consequences of ABA on mesolimbic DA functioning than resilient rats.

There are two important limitations to consider in the interpretation of these results. First, no control conditions were included in the design. A satiated-active control as well as a food restricted-sedentary control condition would have been particularly useful in experiment 2.2.2 examining AMPH-induced locomotion after recovery from ABA. Because these control conditions were omitted, we are limited to concluding that there was no difference between resilient and susceptible rats, but we cannot speak to whether ABA results in long-term changes in mesolimbic DA functioning to begin with. A second limitation of these experiments is that AMPH-induced locomotion was assessed before and after ABA, but not during acute ABA. Thus, it is impossible to determine, based on this design, if mesolimbic DA functioning during acute ABA is associated with ABA susceptibility. Since the completion of this experiment, Foldi, Milton, and Oldfield (2017) have shown that activation of the mesolimbic DA system during ABA increases survival by increasing food intake resulting in attenuated weight loss. It is thus possible that DA hypoactivity occurs in the context of simultaneous food restriction and wheel activity only and can be reversed through activation of the mesolimbic DA pathway.

An important finding from the present experiment is that, once again, that baseline running wheel activity predicted ABA susceptibility whereby susceptible rats displayed higher running wheel activity compared to resilient rats prior to ABA. This finding is consistent with our previous findings in experiment 2.1 as well as other research groups who have made similar observations (e.g., Milton et al., 2018; Perez-Leighton et al., 2014; Pjetri et al., 2012). The replicable ability of baseline running wheel activity to predict ABA susceptibility is particularly important for experimental designs in which susceptible rats need to be identified prior to exposure to ABA. This finding also supports human studies of AN indicating that physical hyperactivity levels may precede AN onset (Davis, Kennedy, Ravelski, & Dionne, 1994; Davis, Blackmore, Katzman, & Fox, 2005; Klein, Mayer, Schebendach, & Walsh, 2007).

Interestingly, although susceptible rats had higher baseline running wheel activity than resilient rats, they did not display more activity in the locomotor activity chambers. This finding supports our observation in experiment 2.1 that susceptible rats did not show more activity in the EPM compared to resilient rats though they were more active on the wheel. Our findings thus far appear to contrast those of Perez-Leighton et al. (2014) and Pjetri et al. (2012) that suggest that spontaneous physical activity, in addition to running wheel activity, predicts ABA susceptibility. In both of their experiments, spontaneous physical activity was monitored in the rats' home cages while the locomotor activity in the present experiments was captured in a novel environment and likely do not reflect spontaneous physical activity in the home cage. It is also possible, however, that running wheel activity is distinct from spontaneous physical activity and serves as a more reliable predictor of ABA susceptibility. Indeed, Novak et al. (2012) suggest that, while running wheel activity and spontaneous physical activity are often correlated, they are distinct behaviours with distinct underlying neurobiological processes. Thinking of susceptible rats as generally more physically active may not only be an oversimplification, but may also mask important distinctions that can be helpful in understanding ABA susceptibility and how it relates to AN.

SUPPLEMENTAL MATERIAL

Table S1.

Descriptive Statistics for the Development of ABA in Experiment 2.2.1.

	Mean (SEM)			
	Resilient		Susceptible	
	Wheel Habituation	Food Restriction	Wheel Habituation	Food Restriction
Food	30.08 (1.23)	9.71 (0.74)	35.13 (2.63)	10.21 (0.94)
Body Weight	244.50 (8.21)	216.46 (9.02)	235.33 (3.33)	200.21 (4.91)
Running Wheel Activity	5704.96 (748.93)	6734.63 (881.09)	12032.17 (651.19)	15189.54 (751.16)

Table S2.*ANOVA Source Table for the Development of ABA in Experiment 2.2.1.*

	<i>F</i>	<i>df</i>	<i>p</i>	η^2
Food Intake				
Phase	303.82	1,10	<.001	0.97
Trait	2.37	1,10	.155	0.19
Phase x Trait	3.05	1,10	.111	0.23
Body Weight				
Phase	466.63	1,10	<.001	0.98
Trait	1.8	1,10	.210	0.15
Phase x Trait	5.87	1,10	.036	0.37
Running Wheel Activity				
Phase	28.82	1,10	<.001	0.74
Trait	54.01	1,10	<.001	0.84
Phase x Trait	7.44	1,10	.021	0.43

Table S3.*Descriptive Statistics for the Development of ABA in Experiment 2.2.2.*

	Mean (SEM)			
	Resilient		Susceptible	
	Wheel Habituation	Food Restriction	Wheel Habituation	Food Restriction
Food	31.07 (2.06)	11.30 (1.27)	31.73 (0.61)	11.17 (1.08)
Body Weight	303.87 (9.43)	271.33 (7.77)	277.07 (4.16)	232.63 (4.69)
Running Wheel Activity	3158.27 (949.76)	5186.19 (1859.41)	9807.97 (756.83)	14194.75 (864.15)

Table S4.*ANOVA Source Table for the Development of ABA in Experiment 2.2.2.*

	<i>F</i>	<i>df</i>	<i>p</i>	η^2
Food Intake				
Phase	963.99	1,10	<.001	0.99
Trait	0.02	1,10	.886	0
Phase x Trait	0.83	1,10	.552	0.04
Body Weight				
Phase	273.55	1,10	<.001	0.97
Trait	23.87	1,10	.001	0.71
Phase x Trait	1.27	1,10	.285	0.11
Running Wheel Activity				
Phase	20.88	1,10	.001	0.68
Trait	26.12	1,10	<.001	0.72
Phase x Trait	2.82	1,10	.124	0.22

EXPERIMENT 2.3. EFFECTS OF OLANZAPINE TREATMENT ON ABA IN RESILIENT AND SUSCEPTIBLE RATS

Despite growing research into the neurobiology of AN, pharmacological interventions are lacking in empirical support for their efficacy beyond relieving depressive and dysmorphic symptoms that typically accompany AN. One of the pharmacological options that has shown the most potential in the treatment of AN is the atypical antipsychotic drug OLZ. OLZ acts as an antagonist on several DA, serotonin, and histamine receptors (Sumiyoshi Tomiki, 2008). Some evidence supporting the use of OLZ in AN include the finding that cognitive-behavioural therapy combined with OLZ is more effective in increasing body weight and reducing activity and anxiety about eating compared to cognitive-behavioural therapy alone (Brambilla et al., 2007). OLZ was also reported to more effectively reduce weight obsession and to increase body weight compared to a placebo in patients with AN (Bissada, Tasca, Barber, & Bradwejn, 2008). Despite these encouraging results, another group was unable to demonstrate OLZ's ability to increase body weight and appetite and a double-blind randomized placebo control study failed to show an effect for OLZ on body weight in adolescents with AN (Kafantaris et al., 2011). These mixed results emphasize the need for a better understanding of individual differences in symptomatology and severity that may result in differential treatment response.

Since the inception of the ABA model in rodents, there have been efforts to either prevent or rescue its development in the hopes of identifying underlying mechanisms that could lead to pharmacological targets for treatment. DA signalling has been at the forefront of efforts with early reports by Routtenberg and Kuznesof (1967) indicating that chlorpromazine, a typical antipsychotic drug, reduced activity and increased food intake during ABA. In more recent years, chronic infusion of OLZ was shown to reduce running wheel activity and increase body weight in rats exposed to ABA (Hillebrand, Koeners, et al., 2005) and treatment with the non-selective DA antagonist, *cis*-flupenthixol, increased food intake in ABA rats in addition to reducing weight loss and activity (Verhagen, Luijendijk, Hillebrand, & Adan, 2009). OLZ treatment in mice exposed to ABA was also found to increase survival and reduce FAA, though it did not alter food intake or overall running wheel activity (Klenotich et al., 2012). Klenotich et al. (2015) later showed that selective antagonism of D2/3 receptors via amisulpride produced larger reductions in weight loss and increases in food intake than OLZ, suggesting that OLZ reduces

ABA symptoms likely through its actions on D2/3 receptors. It would therefore appear that DA antagonism slows the dramatic weight loss associated with ABA primarily via its effect on running wheel activity while its effects on food intake are less consistent.

To the best of our knowledge, ABA resilience and susceptibility have yet to be considered in the OLZ treatment response in ABA. Given our previous finding that baseline running wheel activity predicts ABA susceptibility and therefore plays an important role in accelerated weight loss, it is possible that OLZ treatment would be particularly effective in these rats. Furthermore, if mesolimbic DA activity is involved in ABA susceptibility, one can posit that DA antagonism would rescue the behaviour, making susceptible rats more like resilient rats (i.e., less active and longer survival). Therefore, the goals of the present experiment were to investigate the effects of OLZ treatment in female Sprague Dawley rats undergoing ABA and to examine whether OLZ would be particularly effective in ABA susceptible rats. It was hypothesized that chronic administration of OLZ would increase survival time (i.e., slow weight loss) by decreasing running wheel activity and that this effect would be stronger in susceptible rats compared to resilient rats. Given that the effect of OLZ treatment on food intake in ABA has been more inconsistent, we were interested in assessing whether or not OLZ treatment would increase food intake in ABA.

Method

Subjects

A total of 24 female Sprague Dawley rats (125-150 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec). Due to limitations in equipment availability, two cohorts (12 rats each) were run consecutively and using the same methods and timelines. As in previous experiments, rats were kept on a 12:12 hr reverse light/dark cycle and their body weight, food intake, and water intake was monitored daily at ZT 11-12 throughout the experiment. Upon arrival, rats were individually housed in a colony room and allowed to acclimate for 17 days before being relocated into running wheel cages. With the exception of the restriction phases, rats had *ad libitum* access to both food and water throughout the experiment.

Apparatus and Equipment

Running wheel cages. See “General Methodology” section.

Drugs

OLZ (Sigma-Aldrich) was dissolved in distilled water with 4% glacial acetic acid and adjusted to pH 5 with 1 M NaOH. OLZ or VEH (4% glacial acetic acid adjusted to pH 5 with 1 M NaOH) was continuously infused subcutaneously at a dose of 7.5 mg/kg/day (or 1 μ l/h) using 7-day osmotic minipumps (Alzet, model 2001, DURECT, Cupertino, California). This dose was selected based on previous reports by Hillebrand et al. (2005) that a dose of 7.5 mg/kg/day interfered with the development of ABA in rats. As explained in their paper, chronic administration of OLZ in rats requires infusion concentrations at least 5 times higher than the optimal single dose in humans to achieve clinically comparable D2 receptor occupancy (Hillebrand et al., 2005).

Procedure

The detailed timeline is depicted in Figure 4.12.

Acclimation phase. Rats were individually housed in plastic shoebox cages for 17 days in the animal care facility before being transported to the wheel cages. This acclimation was longer than what was used in our previous experiments (4-7 days) due to logistical complications (the rooms with the running wheel cages were unavailable). During this period, rats' body weight and food intake were monitored daily.

Running wheel habituation phase. Following a 17-day acclimation period in the animal care facility, rats were transferred to the running wheel cages and had unrestricted access to the running wheels, food, and water for 10 days.

Restriction phase 1. On the first day of the restriction phase, food was removed at ZT 13. On the following days, rats had access to food for 1 hr/day between ZT 12-13. The restriction phase was terminated when a rat reached survival criteria (weight loss of 25% of initial body weight) or after a maximum of 12 days (cohort 1) of 15 days (cohort 2).

Recovery phase. Once rats had reached survival criteria or the maximum number of days, they were allowed to recover for 5 days in order to return to their initial body weight.

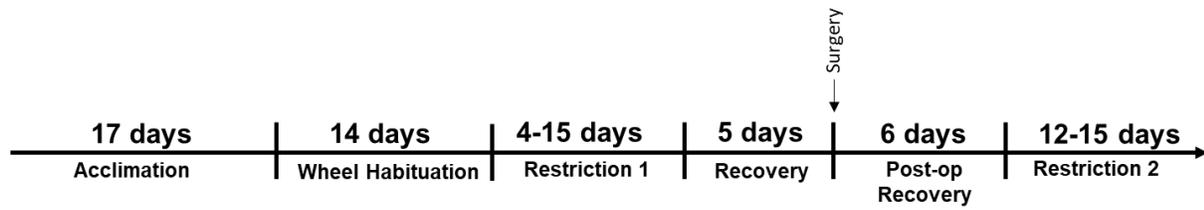


Figure 4.12. Timeline for experiment 2.3.

During this recovery period, rats had *ad libitum* access to food and water with continued access to the running wheels.

Surgery. Following the 5 days of recovery, rats were implanted with subcutaneous Alzet osmotic minipumps (filled with OLZ or VEH) allowing for chronic infusion. Rats were anesthetized with 2% isoflurane for the duration of the procedure and were injected with 2 ml of saline (0.9%) and anafen (0.2 mg/kg). A small area on the rats' backs, between the scapulae, was shaved and disinfected with 70% ethanol and soap. A small incision was made in order to insert the minipump and was closed using silk sutures. Polysporin was applied to the wounds to prevent infections.

Post-Operative Recovery Phase. Following surgery, rats were allowed 6 days to recover during which time they had *ad libitum* access to food and water with continued access to the running wheels. OLZ delivered via minipumps has been reported to begin its actions after 5 days (van der Zwaal et al., 2008). Thus, the 6-day recovery ensured that OLZ would be onboard during the restriction phase. Due to the reported instability of OLZ (van der Zwaal, Luijendijk, Adan, & la Fleur, 2008), 7-day pumps were used and replaced with new pumps every 7 days as described in the surgery details above. Rats underwent 3 to 4 surgeries throughout the experiment.

Restriction phase 2. Six days following surgery, rats began their second restriction phase which followed the same procedure detailed above in restriction phase 1. Rats were euthanized once they reached survival criteria or the maximum days allowed.

Statistical Analyses

As in previous experiments, a median split based on survival time was used to separate the rats into resilient and susceptible groups. Survival time during both restriction phases was analysed using the Kaplan-Meier method and the log-rank test. The data were described using median survival time (i.e., point in time when the cumulative survival drops below 50%). Median survival time was used instead of mean survival time for the following reasons described in more detail by Jager, Van Dijk, Zoccali, and Dekker (2008): 1) survival data are often skewed and the median is a better measure of central tendency for such data; 2) for rats that did not reach survival criteria (i.e., censored cases), it is impossible to know when or whether they would ever

reach survival criteria. Instead, it is only known that a subject has not done so by the end of the observation period which complicates the calculation of a mean. Log-rank tests were used to compare survival curves between groups and tested the null hypothesis that no group differences existed in the probability of survival criteria being reached at any time point.

To characterize changes from the habituation phase to restriction phase, and differences between resilient and susceptible rats, three separate 2 x 2 mixed ANOVAs were used for the following dependent variables: running wheel activity, food intake, and body weight. The between-subjects factor was *trait* (resilient vs. susceptible) and the within-subjects factor was *time* (habituation phase vs. restriction phase 1). The habituation phase represented the average of the dependent variable of interest across the last 4 days of habituation before the start of restriction phase 1. The restriction phase 1 was represented by the average of the dependent variable of interest during the first 4 days of this restriction phase. Partial eta squared was used as a measure of effect size.

Four 3-way mixed ANOVAs were used to examine the effects of OLZ on the following dependent variables: running wheel activity, food intake, body weight, and percentage survival (percentage of total days in which rat had yet to meet starvation criterion). The between-subjects factors were *trait* (resilient vs. susceptible) and *treatment* (VEH vs. OLZ). The within-subjects factor was *time* (restriction phase 1 vs. restriction phase 2). Both restriction phases were represented by the average of the dependent variable of interest across the first 4 days of the respective restriction phase. Partial eta squared was used as a measure of effect size.

To examine the effect of OLZ on survival time during restriction phase 2, two methods were used. To allow for analysis survival duration of both cohorts together, survival duration was standardized as a percentage and a 3-way mixed ANOVA (as described in the previous paragraph) was used. The second method consisted of the Kaplan-Meier method and the log-rank test. Data from cohorts 1 and 2 were analysed separately due to pre-existing differences in survival time between both cohorts (described below). Median survival times were used to describe the data and Log-rank tests were used to compare survival curves between groups.

Results

Data integrity

Two rats were removed from cohort 1. One rat was removed from the experiment due to a post-surgical infection and the other was a statistical outlier (z scores < 3). No rats were removed from cohort 2. The breakdown was as follows: resilient-VEH ($n = 6$), resilient-OLZ ($n = 6$), susceptible-VEH ($n = 5$), and susceptible-OLZ ($n = 5$).

Description of resilient and susceptible rats

A median split of survival time during restriction phase 1 was used to separate the rats into resilient and susceptible groups. Following the median split, the Kaplan-Meier method was used to analyze the survival curves during restriction phase 1 to ensure that the survival differences between the two groups was indeed statistically significant. The log-rank test indicated that, in both experimental cohorts, there was a statistically significant difference in the survival curves of resilient rats, who survived longer, compared to susceptible rats: cohort 1: $\chi^2(1) = 10.35, p = .001$ (Figure 4.13A); cohort 2: $\chi^2(1) = 12.17, p < .001$ (Figure 4.13B). In the first cohort, the median survival time for susceptible rats was 4 days compared to 11 days for resilient rats. Rats in the second cohort survived longer than those in the first cohort, with the median survival time being 6 days for susceptible rats and 12 days for resilient rats. To minimize the risk of a ceiling effect in survival time, it was decided that the restriction phases would be extended to 15 days in cohort 2 (compared to 12 days in cohort 1). Aside from differences in survival time (and therefore in change in body weight), there were no differences in food intake or running wheel activity between cohorts. As such, the running wheel activity, body weight, and food intake data from both cohorts were analysed together and presented below.

Development of ABA

Running wheel activity. As seen in Figure 4.14A, food restriction resulted in a statistically significant increase in running wheel activity whereby average daily wheel rotations was higher during the restriction phase 1 compared to activity during the running wheel habituation phase (*time*: $F(1, 20) = 19.72, p < .001, \eta_p^2 = 0.50$). There was a statistically significant main effect of *trait* whereby susceptible rats had higher average daily wheel rotations compared to the resilient rats (*trait*: $F(1, 20) = 53.09, p < .001, \eta_p^2 = 0.73$). Importantly, the increase in running wheel activity from the habituation phase to the restriction phase was more

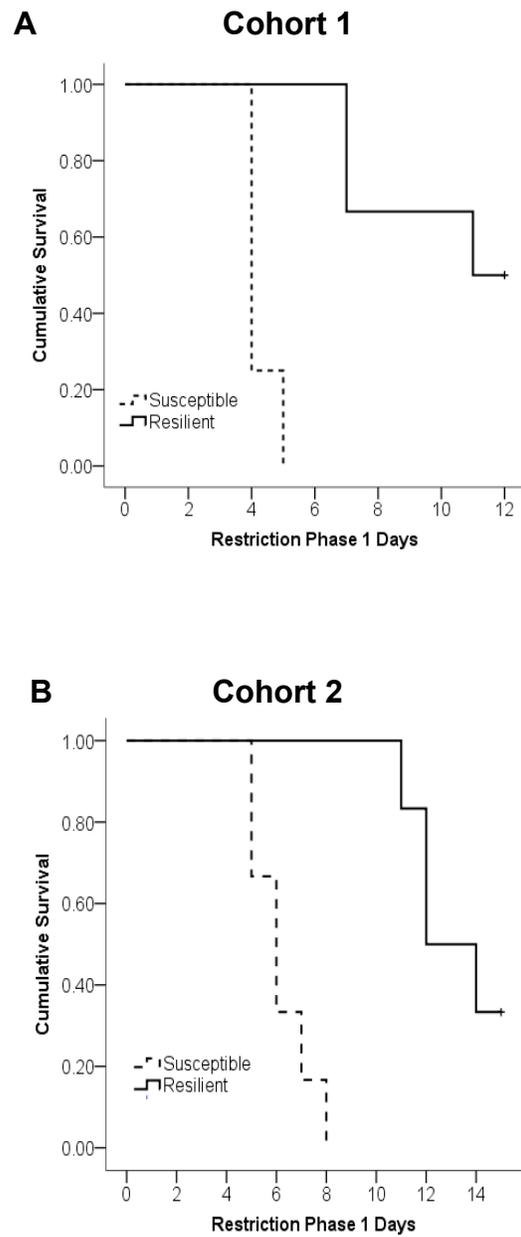


Figure 4.13. Survival curves depicting the cumulative survival of resilient ($n = 6$) and susceptible ($n = 4$) rats in cohort 1 (A) and resilient ($n = 6$) and susceptible ($n = 6$) rats in cohort 2 (B) during restriction phase 1.

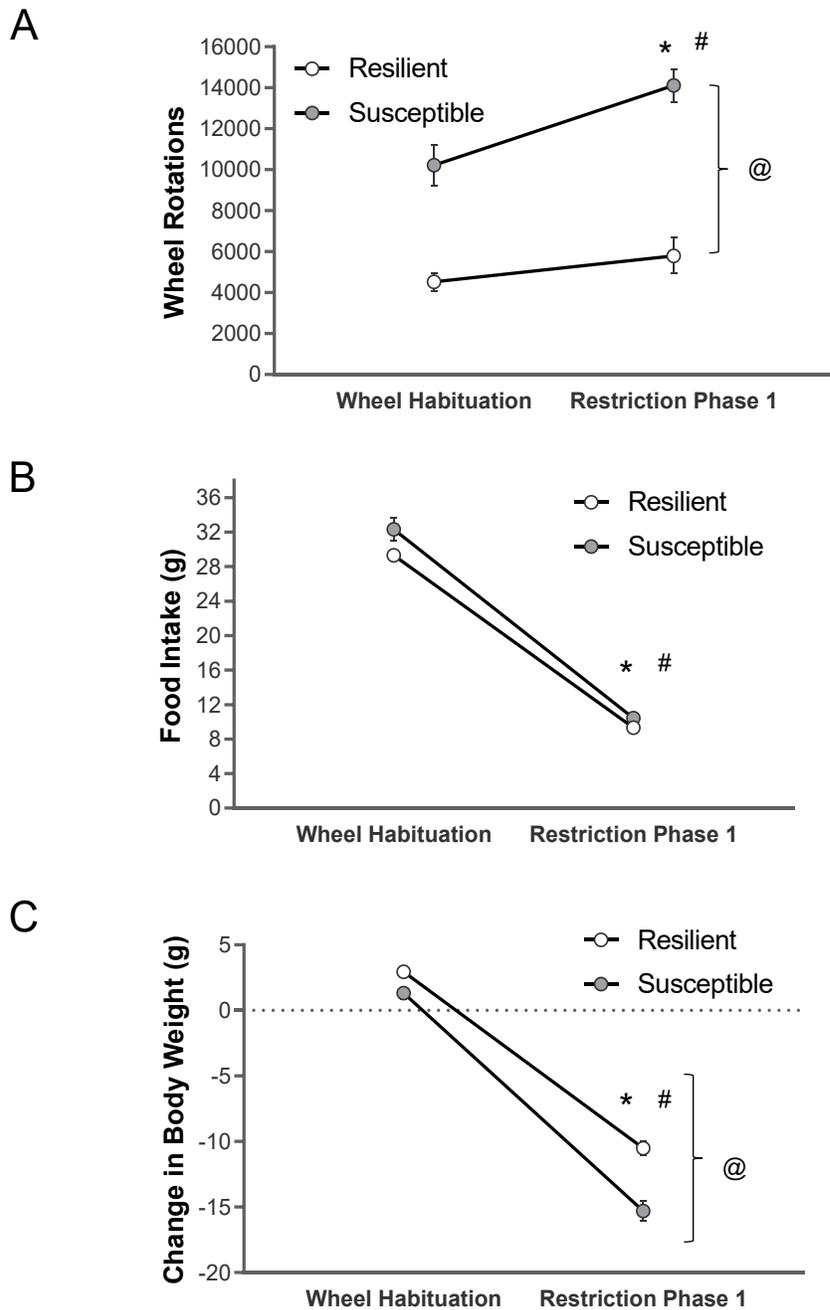


Figure 4.14. The effect of ABA on running wheel activity, food intake, and body weight. (A) Mean running wheel activity during the habituation phase and restriction phase 1 in resilient rats ($n = 12$) and susceptible rats ($n = 10$). * $p < .001$, main effect of *time*; # $p < .001$, main effect of *trait*; @ $p = .036$, *time x trait* interaction. (B) Mean food intake during the habituation phase and restriction phase 1 in resilient rats ($n = 12$) and susceptible rats ($n = 10$). * $p < .001$, main effect of *time*; # $p = .034$, main effect of *trait*. (C) Mean change in body weight during the habituation phase and restriction phase 1 in resilient rats ($n = 12$) and susceptible rats ($n = 10$). * $p < .001$, main effect of *time*; # $p < .001$, main effect of *trait*; @ $p = .010$, *time x trait* interaction.

pronounced for the susceptible rats compared to the resilient rats (*time x trait* interaction: $F(1, 20) = 5.06, p = .036, \eta_p^2 = 0.20$).

Food intake. As expected, rats ate significantly less during the restriction phase compared to the habituation phase (*time*: $F(1, 20) = 1016.60, p < .001, \eta_p^2 = 0.98$; Figure 4.14B). Throughout both phases, susceptible rats ate significantly more than resilient rats (*trait*: $F(1, 20) = 5.20, p = .034, \eta_p^2 = 0.21$). There was no statistically significant *time x trait* interaction ($F(1, 20) = 2.14, p = .159, \eta_p^2 = 0.10$).

Change in body weight. As seen in Figure 4.14C, rats lost significantly more weight during the restriction phase compared to the habituation phase (*time*: $F(1, 20) = 733.89, p < .001, \eta_p^2 = 0.97$). Throughout both phases, susceptible rats lost significantly more weight than resilient rats (*trait*: $F(1, 20) = 48.20, p < .001, \eta_p^2 = 0.71$). A statistically significant *time x trait* interaction indicated that susceptible rats lost more weight than resilient rats during the restriction phase ($F(1, 20) = 8.01, p = .010, \eta_p^2 = 0.29$).

The effects of OLZ on the development of ABA

Running wheel activity. Running wheel activity during the restriction phases is depicted in Figure 4.15A. Overall, rats ran significantly more during restriction phase 1 compared to restriction phase 2 (*time*: $F(1,18) = 9.65, p = .006, \eta_p^2 = 0.35$). A statistically significant main effect of *trait* indicated that susceptible rats ran more than resilient rats (*trait*: $F(1,18) = 30.67, p < .001, \eta_p^2 = 0.63$). The main effect of *treatment* was also statistically significant, indicating that rats receiving OLZ ran less than rats receiving VEH (*treatment*: $F(1,18) = 4.74, p = .042, \eta_p^2 = 0.21$). A statistically significant *time x treatment* interaction indicated that, as expected, the difference in running wheel activity between VEH and OLZ rats appeared to be greater during the restriction phase 2 (when minipumps were onboard; *time x treatment*: $F(1,18) = 14.68, p = .001, \eta_p^2 = 0.45$). This was supported by post-hoc analyses using Bonferroni corrections that revealed that the difference in running wheel activity between treatment conditions was statistically significant during restriction phase 2 only (restriction phase 1: $t(20) = 0.06, p = .952, d = 0.03$; restriction phase 2: $t(20) = 3.05, p = .006, d = 1.30$). In addition, there was a statistically significant *time x trait* interaction indicating that the difference in running wheel activity between resilient and susceptible rats appeared to be diminished during restriction phase

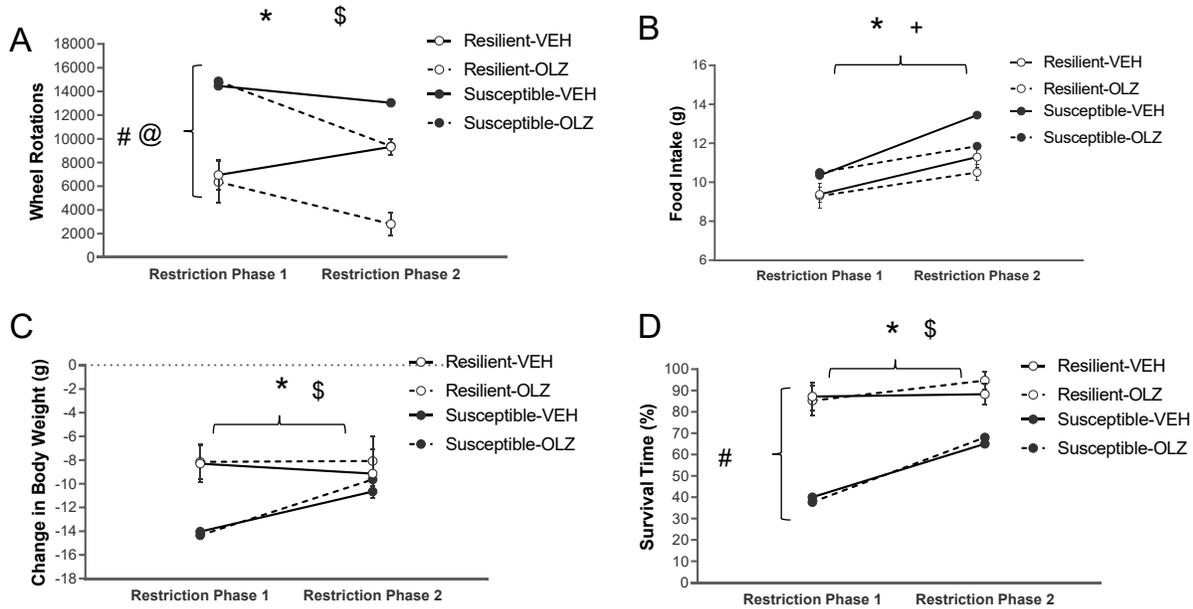


Figure 4.15. The effect of chronic OLZ on running wheel activity, food intake, body weight, and survival time in resilient-VEH ($n = 6$), resilient-OLZ ($n = 6$), susceptible-VEH ($n = 5$), and susceptible-OLZ ($n = 5$) rats. (A) Mean running wheel activity during restriction phase 1 and phase 2. * $p = .006$, main effect of *time*; # $p < .001$, main effect of *trait*; @ $p = .042$, main effect of *treatment*; & $p = .001$, *time x treatment* interaction; \$ $p = .042$, *time x trait* interaction. (B) Mean food intake during restriction phase 1 and phase 2. * $p < .001$, main effect of *time*; + $p = .098$, *time x treatment* interaction. (C) Mean change in body weight during restriction phase 1 and phase 2. * $p = .025$, main effect of *time*; \$ $p = .009$, *time x trait* interaction. (D) Survival time, represented as a percentage of total phase time, during restriction phase 1 and phase 2. * $p = .001$, main effect of *time*; # $p < .001$, main effect of *trait*; \$ $p = .018$, *time x trait* interaction.

2 (*time x trait*: $F(1,18) = 4.81, p = .042, \eta_p^2 = 0.21$). Post-hoc analyses using Bonferroni corrections, however, revealed that susceptible rats showed significantly more running wheel activity compared to resilient rats during both restriction phases (restriction phase 1: $t(20) = -5.98, p < .001, d = -2.56$; restriction phase 2: $t(20) = -2.98, p = .007, d = -1.28$). There was no statistically significant *time x trait x treatment* interaction ($F(1,18) = 0.514, p = .483, \eta_p^2 = 0.03$).

Food intake. Food intake during the restriction phases is depicted in Figure 4.15B. Overall, rats ate significantly more during restriction phase 2 compared to restriction phase 1 (*time*: $F(1,18) = 28.87, p < .001, \eta_p^2 = 0.62$). There were no statistically significant main effects for *trait* or *treatment*, respectively ($F(1,18) = 4.39, p = .051, \eta_p^2 = 0.20$; $F(1,18) = 0.73, p = .403, \eta_p^2 = 0.04$). There was, however, a trending *time x treatment* interaction whereby OLZ-treated rats ate less than VEH-treated rats during restriction phase 2 ($F(1,18) = 3.04, p = .098, \eta_p^2 = 0.15$). The other interactions were neither trending or statistically significant (*trait x treatment*: $F(1,18) = 0.05, p = .835, \eta_p^2 < .01$; *time x trait*: $F(1,18) = 0.88, p = .360, \eta_p^2 = 0.05$; *time x trait x treatment*: $F(1,18) = 0.55, p = .470, \eta_p^2 = 0.03$).

Change in body weight. Change in body weight during the restriction phases can be seen in Figure 4.15C. Overall, rats lost more weight during restriction phase 1 compared to restriction phase 2 (*time*: $F(1,18) = 5.98, p = .025, \eta_p^2 = 0.25$). The main effects for *trait* or *treatment*, respectively, were not statistically significant ($F(1,18) = 3.92, p = .063, \eta_p^2 = 0.18$; $F(1,18) = 0.06, p = .805, \eta_p^2 < 0.01$). A statistically significant *time x trait* interaction suggested that the difference in weight loss between resilient and susceptible rats may have been driven by changes during the first restriction phase (*time x trait*: $F(1,18) = 8.70, p = .009, \eta_p^2 = 0.33$). Indeed, this was supported by post-hoc analyses using Bonferroni corrections indicating that trait differences were only statistically significant during restriction phase 1 (restriction phase 1: $t(20) = 4.04, p < .001, d = 1.73$; restriction phase 2: $t(20) = 0.67, p = .512, d = 0.29$). There were no other statistically significant interactions (*trait x treatment*: $F(1,18) = 0.01, p = .947, \eta_p^2 < .01$; *time x treatment*: $F(1,18) = 0.57, p = .462, \eta_p^2 = 0.03$; *time x trait x treatment*: $F(1,18) = 0.02, p = .894, \eta_p^2 < 0.01$).

Survival time. Survival time during the restriction phases can be seen in Figure 4.15D. Overall, rats survived longer (i.e., higher %) during restriction phase 2 compared to phase 1 (*time*: $F(1,18) = 14.56, p = .001, \eta_p^2 = 0.45$). Collapsed across phases, resilient rats survived

longer than susceptible rats (*trait*: $F(1,18) = 32.76, p < .001, \eta_p^2 = 0.65$). There was a statistically significant *time x trait* interaction ($F(1,18) = 6.72, p = .018, \eta_p^2 = 0.27$). Post-hoc analyses using Bonferroni corrections indicated that the trait difference in survival time was statistically significant during both restriction phases (restriction phase 1: $t(20) = 2.85, p = .010, d = 1.22$; restriction phase 2: $t(20) = 8.67, p < .001, d = 3.71$). The *treatment* main effect and interactions were not statistically significant (*treatment*: $F(1,18) = 0.04, p = .842, \eta_p^2 = 0.01$; *time x treatment*: $F(1,18) = 0.63, p = .439, \eta_p^2 = 0.03$; *trait x treatment*: $F(1,18) = 0.02, p = .883, \eta_p^2 = 0.01$; *time x trait x treatment*: $F(1,18) = 0.03, p = .864, \eta_p^2 = 0.02$).

Survival Analysis.

Cohort 1. Survival curves for rats in cohort 1 can be seen in Figure 4.16. During restriction phase 2, resilient rats continued to survive longer than susceptible rats with a median survival time (collapsing against treatment condition) of 5 days for susceptible rats and 9 days for resilient rats. The median survival time for susceptible rats was 5 days, regardless of whether they were in the VEH or OLZ condition. The median survival time for resilient rats in the OLZ condition was 12 days compared to 9 days for those in the VEH condition. The survival curves for the VEH and OLZ conditions were not significantly different in either susceptible or resilient rats: Resilient rats: $\chi^2(1) = 0.56, p = .456$; Susceptible rats: $\chi^2(1) = 0.43, p = .513$.

Cohort 2. Survival curves for rats in cohort 2 can be seen in Figure 4.17. During restriction phase 2, the median survival time for both resilient and susceptible rats was 14 days. The median survival time for both resilient and susceptible rats in the OLZ condition was 14 days compared to 15 days for rats in the VEH condition. Not surprisingly, the survival curves for the VEH and OLZ conditions were not significantly different in either susceptible or resilient rats: Resilient rats: $\chi^2(1) = 0.80, p = .370$; Susceptible rats: $\chi^2(1) = 0.22, p = .642$.

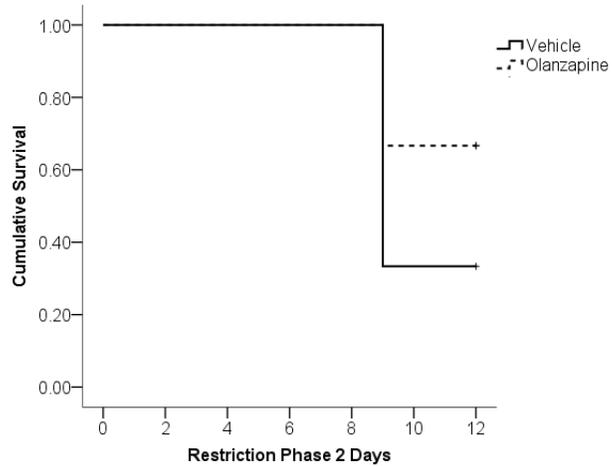
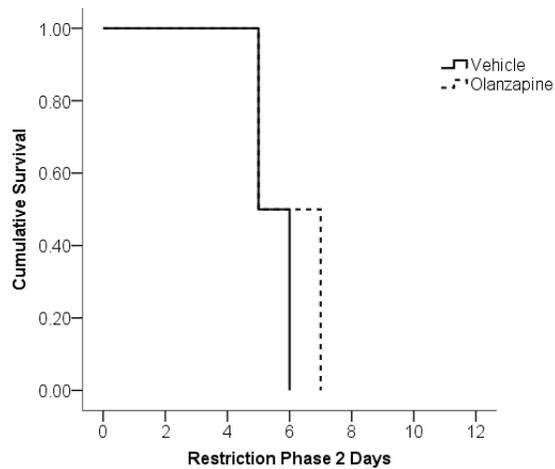
A**Resilient Rats****B****Susceptible Rats**

Figure 4.16. Survival curves depicting the cumulative survival of resilient-VEH ($n = 3$) and resilient-OLZ ($n = 3$) rats (A) and susceptible-VEH ($n = 2$) and susceptible-OLZ ($n = 2$) rats (B) from Cohort 1 during restriction phase 2.

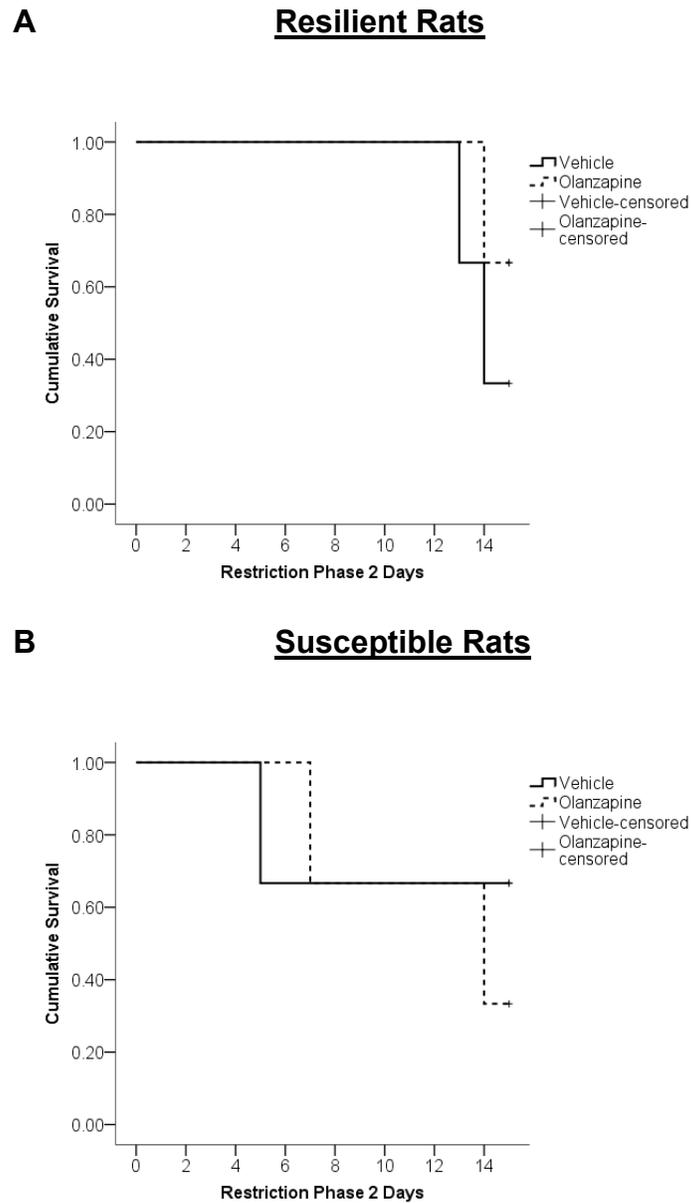


Figure 4.17. Survival curves depicting the cumulative survival of resilient-VEH ($n = 3$) and resilient-OLZ ($n = 3$) rats (A) and susceptible-VEH ($n = 3$) and susceptible-OLZ ($n = 3$) rats (B) from Cohort 2 during restriction phase 2.

Discussion

The main purpose of the present study was to examine whether chronic administration of OLZ during ABA would reduce ABA susceptibility (i.e., prolong survival time) via decreased running wheel activity. We found that OLZ administration decreased running wheel activity for both resilient and susceptible groups, with little to no effect on food intake, weight loss, or survival time. While our results only partially supported our initial hypotheses, they brought to the forefront the importance of studying individual differences in response to ABA.

As in our previous experiments, we found that ABA susceptible rats were more active on the running wheel than resilient rats, not only during ABA, but prior to food restriction thus providing support for the use of baseline running wheel activity for early prediction of ABA susceptibility and resilience. As hypothesized, OLZ treatment resulted in decreased running wheel activity, compared to VEH, for both resilient and susceptible rats. Interestingly, however, OLZ did not result in changes in weight loss or survival time. Moreover, we observed a trending decrease in food intake in OLZ-treated rats compared to VEH (discussed below). While this is not the first report of an effect of OLZ on running wheel activity without statistically significant changes in food intake (e.g., Hillebrand, Van Elburg, et al., 2005; Klenotich et al., 2012), the lack of effect on survival time is surprising. It is possible that OLZ's failure to prolong survival in the present study is due to limitations of the study design employed (e.g., two bouts of ABA) rather than the ineffectiveness of OLZ in interfering with ABA development.

Unlike our previous experiments and those from other researchers examining the effect of OLZ in ABA (e.g., Hillebrand, Van Elburg, et al., 2005; Klenotich et al., 2012), rats in the present study underwent two bouts of ABA. This design allowed us to identify resilient and susceptible rats after a first experience of ABA in order to equally assign rats to the VEH or OLZ condition during the second bout of ABA. Unexpectedly, however, the ABA effect was not as strong during the second bout of ABA. More specifically, we found that susceptible rats, regardless of treatment condition (VEH vs. OLZ), lost less weight, ate more, and survived longer during the second bout of ABA compared to the first, suggesting that individual differences in susceptibility to ABA are less present during a second ABA phase. While this is an interesting finding in and of itself, it interfered with our ability to determine whether OLZ treatment could reduce ABA susceptibility as the rats no longer appeared to be susceptible. Our observation that

individual differences in ABA response seem to be lost after a first round of ABA warrants further investigation as it is in contrast with what has been reported in mice whereby exposing mice to two bouts of ABA not only captures the relapsing nature of AN, but also increases individual differences allowing for the study of resilience and susceptibility (Chowdhury, Wable, Sabaliauskas, & Aoki, 2013). One possible explanation is that surgery and the presence of the minipumps may have interfered with the development of ABA. It should be noted, however, that surgery and the potential discomfort of the minipumps does not explain the reduction in running wheel activity as this reduction was not observed in VEH rats who experienced the same procedure. Another factor to consider is the age of the rats at the time of the second bout of ABA. Due to logistical reasons that were out of our control, the rats in the present experiment arrived at approximately PD60 and had to be kept in the animal care facility for 17 days before being moved to the wheel cages and beginning the experiment – a time that is substantially longer than the 4-7 days of acclimation that has been used in all our previous experiments. Consequently, rats were approximately PD110 at the time of the second ABA phase. Age has been shown to effect ABA development whereby ABA develops were rapidly and reliably in younger rats (Frintrop et al., 2018).

Should the present experiment be repeated, some adjustments to the design should be considered. For instance, it would be advisable to use baseline running wheel activity to identify rats that are most likely susceptible to ABA in order to assess the effects of OLZ on a first experience of ABA, which, based on our data, seems to be most severe in adult female Sprague Dawley rats. Another limitation of the present study was that the ABA phases were ended before all rats – resilient rats specifically – reached starvation criteria. This was problematic as it may have resulted in a ceiling effect in survival duration for resilient rats rendering it impossible to determine if OLZ increased survival in these rats. It remains to be examined whether prolonging the ABA phase would have resulted in some of the resilient rats eventually meeting starvation criterion and determining whether or not OLZ would then have delayed starvation. It is also possible, however, that resilient rats would have maintained their body weight and thus never reached starvation criterion regardless of the duration of ABA. Finally, another major limitation of the present study was the small sample size and the fact that the experiment was carried out across two cohorts at different times. While this limitation is not always avoidable for logistic

reasons, as was the case here, it would be ideal to conduct this experiment at one time with a larger number of rats.

It is worth reiterating that important differences were observed between the two experimental cohorts whereby the first cohort reached the starvation criterion much more rapidly than the second cohort during their first ABA phase. In other words, the first cohort was generally more “susceptible” than the second cohort. The decision was therefore made to prolong the ABA phase in the second cohort that was more resilient to allow for individual differences to be observed. This unfortunately meant that survival analyses had to be run separately for each cohort, unlike the rest of the analyses, because the duration of the ABA phase was different. Curiously, no additional differences were found between the two cohorts other than survival time (i.e., no cohort differences in food intake or running wheel activity). It should also be noted that the rats were ordered from the same facility, were the same age, and were tested in the same room and apparatus. While this discrepancy between our two cohorts complicated the analyses and may cloud the results, it serves as an important reminder of the individual differences that do exist between rats with regards to ABA susceptibility and the importance of furthering our understanding of the underlying mechanisms involved in ABA susceptibility.

Despite the complications and limitations of the present study, the main finding that chronic OLZ reduced running wheel activity is an important one given that the most consistent difference between resilient and susceptible rats at the moment seems to be hyperactivity. Given that OLZ acts on DA, serotonin, and histamine receptors, the specific mechanism involved in reducing running wheel activity in ABA is unknown. Since the completion of the present experiment, Foldi et al. (2017) demonstrated that activation of the VTA-NAcc pathway (largely dopaminergic) in rats exposed to ABA promoted food intake and prolonged survival, with no effect on overall running wheel activity. This finding, paired with our observation of a trending decrease in food intake in rats receiving OLZ, lends support to the suggestion that hypofunction of the mesolimbic DA system plays a role in ABA. Importantly, however, Foldi et al., (2017)’s observation that VTA-NAcc activation did not change running wheel activity suggests that the effect of OLZ on running wheel activity observed in the present experiment is likely not due to DA antagonism in the mesolimbic pathway. This is further supported by the finding that extracellular DA release in the NAcc increases during feeding but not during periods of

hyperactivity in ABA (Verhagen, Luijendijk, Korte-Bouws, et al., 2009). It is thus possible that OLZ's actions on other DA pathways, serotonin, histamine, or a complex interaction of actions on these different systems is involved in reducing hyperactivity in ABA.

Future studies with improved designs and larger sample sizes will be necessary to clarify whether or not OLZ reduces ABA susceptibility beyond its effects on running wheel activity. Furthering our understanding of the mechanisms involved in ABA susceptibility may eventually inform more targeted interventions. Given that hyperactivity is observed only in a subset of individuals with AN, pharmacological interventions that reduce hyperactivity, such as OLZ, may be most effective when administered to individuals with this relevant symptom profile. This is supported by the encouraging results from a clinical study in adolescents with AN that showed that OLZ treatment was particularly effective in reducing hyperactivity which correlated with improvements in body weight and that these improvements continued to increase in the following 5 months to follow-up (Leggero et al., 2010).

CHAPTER 4 SUMMARY

In *experiment 2.1*, we assessed baseline anxiety-like and depression-like behaviours using the EPM, FST, and SPT prior to exposure to the ABA protocol. This was the first experiment in which a median split based on percent of initial body weight towards the end of ABA was used to retrospectively identify resilient and susceptible rats. Consistent with previous reports, we found that susceptible rats showed higher baseline running wheel activity suggesting that activity prior to ABA could serve as a reliable predictor of ABA susceptibility. Of the three behavioural paradigms used, resilient and susceptible rats differed in the FST only. More specifically, susceptible rats showed longer latency to immobility and less immobility time (typically interpreted as less depression-like behaviour).

In *experiment 2.2*, locomotor activity following varying doses of AMPH was used as an index of mesolimbic DA activity. These tests took place following ABA exposure and, in a different set of rats, prior to ABA to assess for potential baseline differences between resilient and susceptible rats. No differences were observed between resilient and susceptible rats in response to the AMPH challenges, suggesting that there are no trait differences in mesolimbic DA activity at baseline or following exposure to ABA. An important limitation of this study was that no non-ABA control group was used making it impossible to determine whether or not a history of ABA results in changes in mesolimbic DA function. As in experiment 2.1, we found that baseline running wheel activity predicted ABA susceptibility. These results suggest that the higher baseline wheel activity in susceptible rats is not related to differences in mesolimbic DA.

Finally, in *experiment 2.3*, rats were administered chronic OLZ during ABA to examine whether OLZ would prolong survival, particularly in susceptible rats. Here again, baseline running wheel activity was found to predict ABA susceptibility. This replicable finding is important for studies in which susceptibility needs to be identified prior to a manipulation. Rather than using baseline activity to assign rats to groups, rats in the present study underwent two phases of ABA – a first to identify susceptibility and a second to test OLZ treatment. Unfortunately, we found that all rats, regardless of treatment, showed an attenuated ABA response during the second bout. This interfered with our ability to draw conclusions about OLZ treatment. We did find, however, that OLZ reduced running wheel activity in resilient and

susceptible rats. It is unclear whether this led to prolonged survival due to complications discussed in the experiment discussion which resulted in decreased statistical power.

**CHAPTER 5: DIFFERENCES IN NEURONAL ACTIVATION AND IMPULSIVITY
BETWEEN ABA-RESILIENT AND ABA-SUSCEPTIBLE RATS**

ABSTRACT

When exposed to ABA, some rats show increased susceptibility to the activity-based anorexia (ABA) procedure whereby they show higher hyperactivity in response to food restriction and rapid weight loss compared to more resilient rats that manage to maintain their body weight. The two experiments presented here were designed to further explore the differences between resilient and susceptible rats by focusing on brain activity and impulsivity. Adult female Sprague Dawley rats were used in both experiments. All rats were exposed to two bouts of ABA during which feeding was limited to 60 min/day and they had continuous wheel access. In experiment 3.1, brains were collected on the last day of the second bout of ABA. Using c-Fos immunohistochemistry, we examined whether differences in neuronal activity were present between resilient and susceptible rats in the prefrontal cortex (PFC), nucleus accumbens (NAcc), hypothalamus, amygdala, and the granular insular cortex. These regions were selected based on previous studies in anorexia nervosa (AN) and ABA as well as their involvement in hunger, feeding, homeostasis, reward processing, and cognitive functioning. Susceptible rats showed a trend for more neuronal activity in the prelimbic cortex and infralimbic cortex of the PFC and lower activity in the NAcc shell compared to resilient rats, though these differences did not reach statistical significance. No differences were observed in the other sampled brain regions. These results are in line with studies in AN showing enhanced neural activity in the PFC leading to heightened cognitive control and reduced reward functioning. To build on these findings, response inhibition, a facet of impulsivity that relies on the PFC, was assessed in resilient and susceptible rats using a Go/No-Go task. Rats were trained before ABA and tested after two bouts of ABA. Depression- and anxiety-like behaviours were also assessed using the forced swim test (FST) and elevated-plus maze (EPM), respectively, before and after ABA. No differences were observed between resilient and susceptible rats, and pre- and post-ABA on the EPM or the Go/No-Go task. Immobility time in the FST was higher after two bouts of ABA compared to before, though no trait differences were observed. This suggests that the experience of ABA may increase depression-like behaviours though the absence of a non-ABA control condition limits this interpretation. Importantly, and as in previous experiments, rats adjusted their behaviour in a second bout of ABA thereby diminishing the individual differences in ABA response and may partially explain why we failed to observe significant differences in neural activity, FST, and the Go/No-Go task between resilient and susceptible rats. It should be noted that we also attempted

to investigate the effect of inhibiting the PFC using DREADD technology. Due to the preliminary nature of these data, they are not presented here but can be found in Appendix 2 and will be considered in the general discussion.

EXPERIMENT 3.1. NEURONAL ACTIVITY IN ABA RESILIENT AND SUSCEPTIBLE RATS

Advances in human brain imaging have made it possible to study the brain of individuals with AN. Neuroimaging studies using computed tomography have typically reported cerebral atrophy and enlarged ventricles in acute AN (Dolan, Mitchell, & Wakeling, 1988; Heinz, Martinez, & Haenggeli, 1977; Nussbaum, Shenker, Marc, & Klein, 1980). In line with these findings, MRI studies in AN have shown larger CSF volumes in association with deficits in both total grey matter and total white matter volumes (Katzman et al., 1996). Results following weight restoration have been inconsistent, however, with studies showing persistent alterations and others showing normalization after recovery (reviewed in Kaye, 2008). In terms of regional specificity, abnormal MRI activity in response to food images has been reported in several brain regions of individuals with AN such as the insula, orbitofrontal cortex, mesial temporal, parietal, and anterior cingulate cortex (Kaye, 2008). There has been a strong focus on serotonin and DA neurotransmitter function in limbic and executive pathways in both the ill state and following recovery. Serotonergic abnormalities have been reported in AN in regions including the amygdala, insula, central striatum, anterior cingulate cortex and the PFC and is thought to play a role in symptoms including dysfunctional appetitive regulation (Leibowitz & Shor-Posner, 1986), anxiety, behavioural inhibition, and self-control (Higley & Linnoila, 1997; Lucki, 1998; Soubri , 1986). Alterations in the DAergic system functioning have also been observed including, but not limited to, increased binding of D2/D3 receptors in the anteroventral striatum, a region that contributes to optimal responses to reward stimuli (Frank et al., 2005). Striatal DA transmission dysfunction in AN might also contribute to altered affect, decision-making, executive control, motor activity, and decreased food ingestion in AN (Yin & Knowlton, 2006).

The ABA model in rodents has been used to compliment human research and further our understanding of brain areas and neurocircuits that may contribute to AN-like symptoms, though few of these studies have specifically examined resilience and susceptibility to ABA development. Wable et al. (2014) found that mice exposed to ABA displayed elevated levels of GABA receptor alpha4 subunits on GABA interneurons near excitatory synapses in the amygdala which they posit increases excitability of the amygdala which in turn increases anxiety and ABA response. The hypothalamus has also received attention with its important implication

in the regulation of hunger, satiety, energy metabolism and body weight. One study found that FAA in the running wheel was correlated with c-Fos expression in the dorsomedial hypothalamus (Verhagen, Luijendijk, de Groot, et al., 2011). Verhagen, Luijendijk, Korte-Bouws, Korte, and Adan (2009) used microdialysis to quantify DA release in the NAcc, an area involved in feeding behaviour and reward, in rats exposed to ABA and found that DA release was increased in the NAcc during feeding behaviour but not during FAA, suggesting that DA in the NAcc does not trigger hyperactivity (or, more specifically, FAA) in the early stages of ABA development.

In the present experiment, we aimed to contribute to the understanding of ABA resilience and susceptibility by examining where differences in brain activity may occur between the two subsets of rats following two bouts of ABA. We surveyed several relevant brain areas using c-Fos immunohistochemistry as a nonspecific marker of neuronal activity. Briefly, changes in neuronal activity lead to second messenger signaling cascades that induce the expression of the immediate early gene *c-fos* which in turn induces the production of the transcription factor c-Fos. The c-Fos protein can be detected using immunohistochemical techniques 20-90 minutes after neuronal excitation (Bullitt, 1990). Using c-Fos immunohistochemistry, we examined whether differences in neuronal activity were present between ABA resilient and susceptible rats in brain regions involved in hunger, feeding, regulation of the body's homeostasis, emotional and reward processing, and cognitive functions. These regions included the PFC, NAcc, hypothalamus, amygdala, and granular insular cortex. Brain tissue of these areas of interest was collected at the end of the second ABA phase in the hours following feeding (ZT 14-17) and therefore corresponded to the postprandial period. This time window was selected based on previous reports that postprandial running wheel activity, rather than FAA, is positively correlated with weight loss (Wu et al., 2014) as well as unpublished findings from our laboratory suggesting that ABA susceptibility may be associated with higher PPA but not FAA (see Appendix 3).

Method

Subjects

Female Sprague Dawley rats ($n = 12$; 125-150 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec) and housed in a colony room on a 12:12 hr reverse light/dark cycle. Upon arrival, rats were initially pair-housed in plastic shoebox cages and were

then separated into individual shoebox cages on day 3. After 7 days of acclimation in the animal care facility, rats were transferred to the laboratory where they were permanently housed in running wheel cages inside sound-attenuating boxes until the end of the experiment. With the exception of the restriction phases, rats had *ad libitum* access to both food and water. Body weight (g), food intake (g), and water intake (g) was monitored daily at ZT 11-12.

Apparatus

Running Wheel Cages. See “General Methodology” section.

Procedure

The detailed timeline is depicted in Figure 5.1.

Wheel habituation phase. Seven days following arrival, rats were transferred from the animal care facility to the individual running wheel cages where they were permanently housed for the remainder of the experiment. During this phase, all rats had *ad libitum* food and water and continuous access to the running wheel. This phase lasted 14 days.

Restriction Phase 1. Following the wheel habituation phase, the first restriction phase began. Food was removed at ZT 13. On the following days, rats had access to food for 1 hr/day between ZT 12-13. This restriction phase lasted 4 days.

Recovery Phase. Following restriction phase 1, rats recovered for 6 days. During this recovery period, rats had *ad libitum* access to food and water with continued access to the running wheels.

Restriction Phase 2. Following the 6-day recovery, the second restriction phase began using the same procedure as in restriction phase 1. This restriction phase lasted 6 days.

Perfusions and tissue preparation. On the 6th day of restriction phase 2 between ZT 14-17 (postprandial phase), rats were overdosed with sodium pentobarbital and transcardially perfused with PBS followed by 4% paraformaldehyde. Brains were extracted, and post-fixed for 24 hours, cryoprotected with 30% sucrose at 4°C for 48 hours and stored in -80°C. Coronal sections (40 µm) were sliced on a Leica cryostat. Slices were stored at -20°C in cyroprotectant.



Figure 5.1. Timeline for experiment 3.1.

Fos immunohistochemistry. Free-floating brain sections containing the regions of interest were washed 12 x 5 minutes in TBS. Sections were then blocked in 3% normal goat serum and 0.20% Triton-X in TBS for 2 hours at 4°C. Sections were then incubated for 48 hours at 4°C with the primary rabbit anti-Fos antibody (Cell Signalling #2250S; 1:2000), in 3% Normal Goat Serum and 0.15% Triton-X in TBS. Following primary incubation, sections were washed 5 x 5 minutes in TBS and then quenched in a 0.3% TBS hydrogen peroxide 30 minutes at 4°C. Following quenching, sections were washed 5 x 5 minutes and were then incubated in the secondary antibody solution containing biotinylated goat anti-rabbit IgG antibody (Vector Laboratories; 1:200), 3% normal goat serum and 0.2% Triton-X at 4°C for 1 hour. Following secondary incubation, sections were washed 3 x 5 minutes in TBS, and were then incubated for 1 hour in a Vectastain Elite ABC solution (Vector Laboratories) at 4°C. Sections were washed 3 x 5 minutes in TBS. Next, Fos reactive cells were visualized by reacting with DAB and Nickle Cl (Vector Laboratories) for 1.5 minutes. The reaction was briefly paused by placing the sections in TBS. The reaction was stopped by placing the sections in tap water for 5 minutes. Sections were washed 3 x 5 minutes in TBS and mounted onto SuperFrost Plus microscope slides and, cover-slipped with Permount medium (Fisher Scientific).

Fos immunoreactivity quantification. Fos immunoreactivity (IR) was assessed by an experimenter blind to conditions. Images were taken using the software program ToupView (Hangzhou ToupTek Photonics Co., Ltd.) that was connected to a ToupTek LCMOS digital camera and a Leica microscope (DM4000). ImageJ software (National Institute of Health) was used for Fos-labeled cell counting. One image per section was taken at 20x objective. For each subject, counts from three bilateral sections with the highest number of Fos-IR cells were averaged from the following brain regions: NAcc shell and core (bregma: AP +1.44 to AP +2.28), GIC (bregma AP +1.68 to AP -0.84), PrL, IL, and CgL (bregma AP +4.20 to AP +2.52), CA and BLA (bregma AP -1.56 to AP -2.92), and LH (bregma AP -1.56 to AP -2.76).

Statistical Analysis

A median split on percent of initial body weight after two bouts of ABA was used to retrospectively assign rats to the resilient or susceptible groups whereby rats with the lowest percent body weight (i.e., those that lost the most weight during ABA) were considered

susceptible. These two groups were then used as categorical independent variables in a series of ANOVAs and *t*-tests.

To characterize changes from the wheel habituation phase to the restriction phase in resilient and susceptible rats, three separate 2 x 2 mixed ANOVAs were used for the following dependent variables: running wheel activity, food intake, and change in body weight. The between-subjects factor was *trait* (resilient vs. susceptible) and the within-subjects factor was *phase* (habituation phase vs. restriction phase). The habituation phase represented the average across the last 4 days of habituation before the start of restriction phase for each of the dependent variables of interest. The restriction phase was represented by the average across the 4 days of restriction phase 1 for each of the dependent variables of interest.

To characterize the changes from restriction phase 1 to restriction phase 2 the same mixed 2 x 2 ANOVAs described above were used for the same three dependent variables. Both restriction phases 1 and 2 were represented by the average across the first 4 days of the respective phases for each of the dependent variables of interest.

To analyse c-fos-IR, 9 independent-samples *t*-tests were used to test for differences between resilient and susceptible rats in each of the 9 samples brain areas. Trait was used as the independent variable and mean c-fos-IR (adjusted for image area) was used as the dependent variable. The option of using a MANOVA to analyse these results was carefully considered. In designs such as this one, MANOVA can be preferable over multiple ANOVAs or *t*-tests as it combines the values into a weighted linear composite and thus takes into account the relationships between them. Ultimately, we opted for several *t*-tests rather than one MANOVA for two reasons. First, Meyers, Gamst, and Guarino (2013) advise against using a MANOVA when dependent variables are relatively uncorrelated. The value of creating a weighted linear composite in MANOVA is that it takes into account correlations between the dependent variables, allowing them to join forces. The authors suggest that a significant Bartlett's test of sphericity is indicative of sufficient correlation between the dependent variables to proceed with the MANOVA. In our case, Bartlett's test was not statistically significant indicating, according to the above, that the dependent variables were not sufficiently correlated for a MANOVA. Secondly, Meyers, Gamst, and Guarino (2013) explain that MANOVA requires larger sample sizes than independent ANOVAs or *t*-tests because of the additional burden of analyzing

simultaneously multiple dependent measures. They suggest a minimal sample size requirement for a MANOVA is that the number of cases per group exceeds the number of dependent variables. The authors also provide a more realistic minimum sample size of at least 20 cases per group to achieve minimal levels of statistical power. In the present experiment, we had 9 dependent variables versus only 5 resilient rats and 6 susceptible rats. As such, we opted to analyse the brain regions separately in individual *t*-tests with a Bonferroni correction to avoid alpha-level inflation.

Results

Data Integrity

A total of 12 rats were used in this experiment. One brain was damaged during slicing and was removed from the analyses resulting in 5 rats in the resilient group and 6 rats in the susceptible group.

Development of ABA

Running wheel activity. As seen in Figure 5.2A, susceptible rats' running wheel activity was significantly higher than that of the resilient rats (*trait*: $F(1, 9) = 138.97, p < .001, \eta_p^2 = 0.94$). Across all rats, food restriction resulted in a statistically significant increase in running wheel activity whereby average daily wheel rotations was higher during the restriction phase 1 compared to activity during the running wheel habituation phase (*phase*: $F(1, 9) = 17.55, p = .002, \eta_p^2 = 0.66$). Importantly, the increase in running wheel activity from the habituation phase to the restriction phase was more pronounced for the susceptible rats compared to the resilient rats, as indicated by the statistically significant *phase x trait* interaction ($F(1, 9) = 9.13, p = .014, \eta_p^2 = 0.50$).

Food intake. As expected, rats ate significantly less during the restriction phase compared to the habituation phase (*phase*: $F(1, 9) = 527.48, p < .001, \eta_p^2 = 0.98$; Figure 5.2B). Collapsed across phases, susceptible rats ate significantly more than resilient rats (*trait*: $F(1, 9) = 6.39, p = .032, \eta_p^2 = .42$). Importantly, this difference may be driven by a trending *phase x trait* interaction suggesting that the trait difference was more pronounced during the wheel habituation phase compared to the restriction phase 1 (*phase x trait*: $F(1, 9) = 16.78, p = .087, \eta_p^2 = 0.29$).

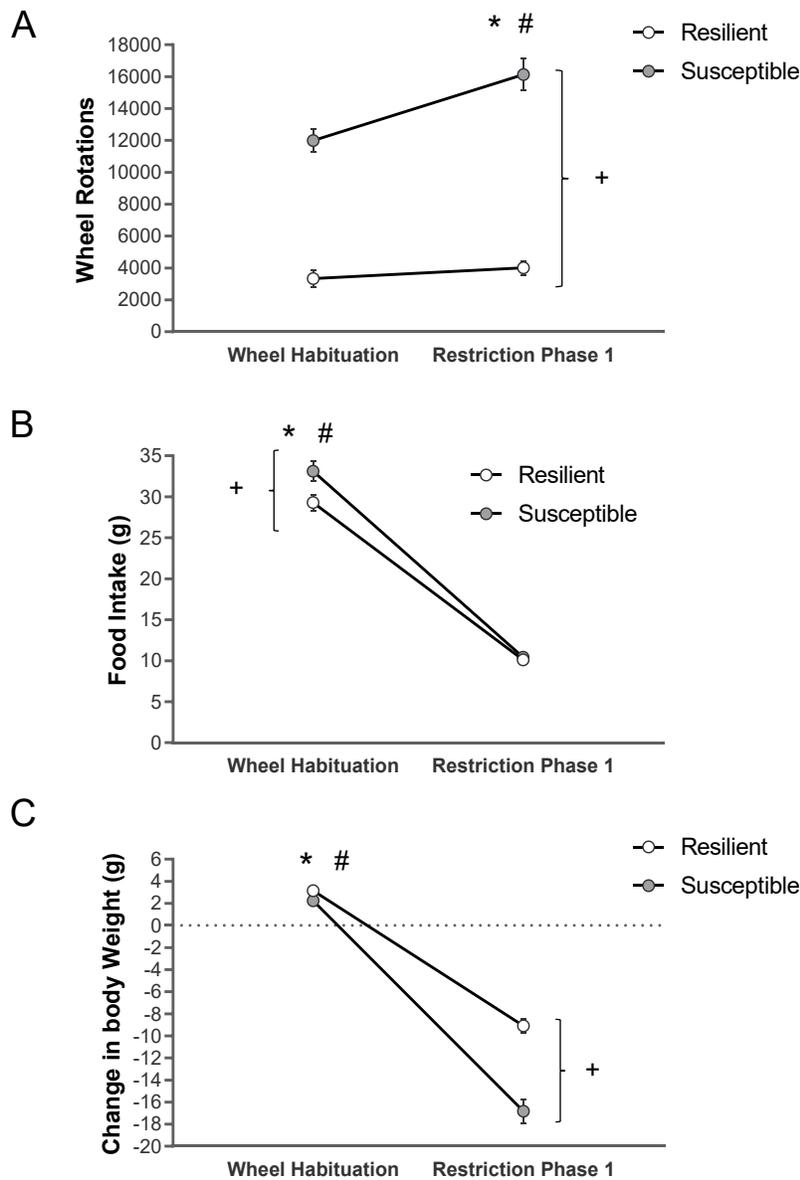


Figure 5.2. The effect of food restriction on running wheel activity, food intake, and body weight in ABA-resilient ($n = 5$) and ABA-susceptible rats ($n = 6$). (A) Mean running wheel activity during the habituation phase and restriction phase 1. $*p < .001$, main effect of *trait*; $\#p = .002$, main effect of *phase*; $+p = .014$, *phase x trait* interaction. (B) Mean food intake during the habituation phase and restriction phase 1. $*p = .032$, main effect of *trait*; $\#p < .001$, main effect of *phase*; $+p = .087$, trending *phase x trait* interaction. (C) Mean change in body weight during the habituation phase and restriction phase 1. $*p < .001$, main effect of *trait*; $\#p < .001$, main effect of *phase*; $+p = .001$, *phase x trait* interaction.

Change in body weight. As seen in Figure 5.2C, rats lost significantly more weight during the restriction phase compared to the habituation phase (*phase*: $F(1, 9) = 458.29, p < .001, \eta_p^2 = 0.98$). Overall, susceptible rats lost significantly more weight than resilient rats (*trait*: $F(1, 9) = 47.98, p < .001, \eta_p^2 = .84$). Importantly, the change in body weight upon food restriction was more severe in susceptible rats compared to resilient rats (*phase x trait*: $F(1, 9) = 21.80, p = .001, \eta_p^2 = 0.71$).

Comparison two bouts of ABA

Running wheel activity. Running wheel activity during both restriction phases can be seen in Figure 5.3A. Running wheel activity was significantly lower during the second restriction phase compared to the first (*phase*: $F(1, 9) = 11.60, p = .008, \eta_p^2 = 0.56$). Susceptible rats ran significantly more than resilient rats overall (*trait*: $F(1, 9) = 150.89, p < .001, \eta_p^2 = 0.94$). Importantly, the decrease in running wheel activity from the first to the second restriction phase was most apparent in susceptible rats (*phase x trait*: $F(1, 9) = 4.92, p = .054, \eta_p^2 = 0.35$).

Food intake. Food intake during both restriction phases can be seen in Figure 5.3B. Overall, food intake was significantly higher during the second restriction phase compared to the first (*phase*: $F(1, 9) = 34.23, p < .001, \eta_p^2 = 0.79$) and susceptible rats ate significantly more than resilient rats (*trait*: $F(1, 9) = 16.49, p = .003, \eta_p^2 = 0.65$). The overall increase in food intake from restriction phase 1 to restriction phase 2 was driven by the susceptible rats (*phase x trait*: $F(1, 9) = 56.00, p < .001, \eta_p^2 = 0.86$).

Change in body weight. Change in body weight during both restriction phases can be seen in Figure 5.3C. Susceptible rats lost more weight than resilient rats overall (*trait*: $F(1, 9) = 33.30, p < .001, \eta_p^2 = 0.79$). Rats appeared to lose less weight during the second restriction phase compared to the first, though this difference did not reach statistical significance (*phase*: $F(1, 9) = 3.59, p = .091, \eta_p^2 = 0.29$). There was no significant interaction (*phase x trait*: $F(1, 9) = 0.07, p = .797, \eta_p^2 = 0.01$).

Fos immunohistochemistry

A representative image of c-fos-IR can be seen in figure. 5.4.

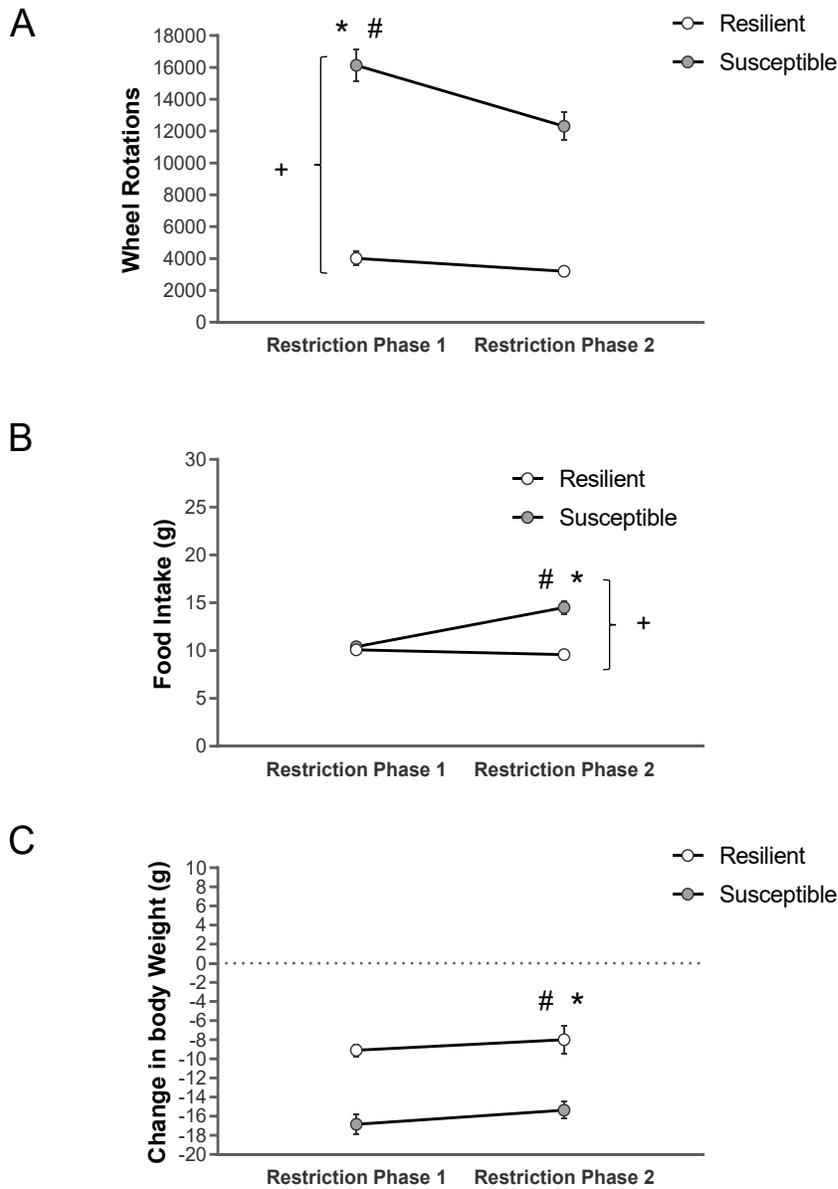


Figure 5.3. Differences in running wheel activity, food intake, and body weight in resilient rats ($n = 5$) and susceptible rats ($n = 6$) across two bouts of ABA. (A) Mean running wheel activity during the restriction phase 1 and restriction phase 2. $*p < .001$, main effect of *trait*; $\#p = .008$, main effect of *phase*; $+p = .054$, *phase x trait* interaction. (B) Mean food intake during the restriction phase 1 and restriction phase 2. $*p = .003$, main effect of *trait*; $\#p < .001$, main effect of *phase*; $+p < .001$, *phase x trait* interaction. (C) Mean change in body weight during the restriction phase 1 and restriction phase 2. $*p < .001$, main effect of *trait*; $\#p = .091$, trending main effect of *phase*.

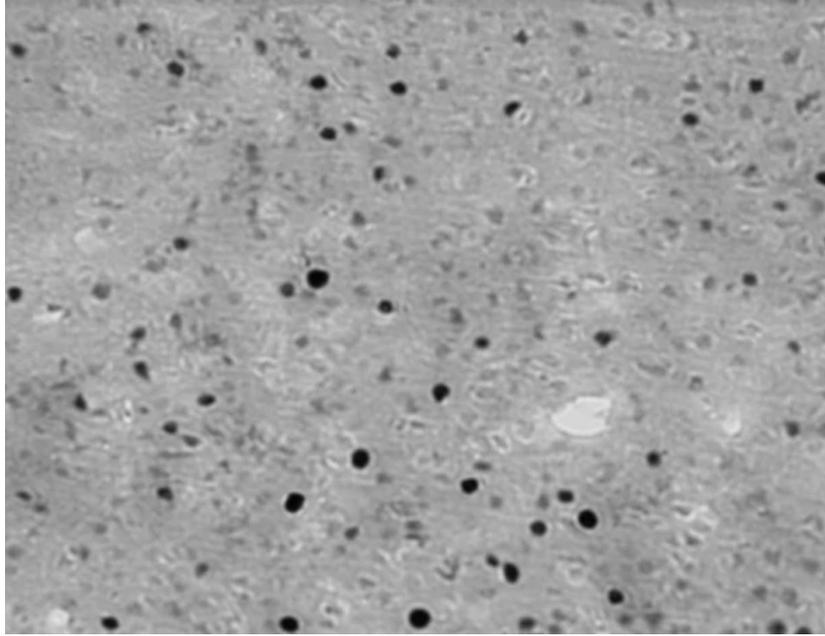


Figure 5.4. Example of Fos immunoreactivity from a representative image used for fos-labeled cell counting. This image of the infralimbic cortex of a susceptible rat was taken at 20x objective.

Prefrontal Cortex. c-fos-IR count in different areas of the PFC can be seen in Figure 5.5A. Susceptible rats appeared to have higher c-fos staining in both the prelimbic cortex and infralimbic cortex, though these differences did not reach statistical significance (prelimbic cortex: $t(6.56) = -2.07, p = .088, d = 1.16$; infralimbic cortex: $t(9) = -1.88, p = .093, d = 1.14$). There were no statistically significant differences between resilient and susceptible rats in c-fos staining in the cingulate cortex ($t(6.29) = -1.52, p = .177, d = 0.85$).

Nucleus Accumbens. c-fos-IR in the NAcc core and shell can be seen in Figure 5.5B. Susceptible rats appeared to have lower c-fos-IR in the shell compared to resilient rats, though this difference did not reach statistical significance ($t(6.50) = 2.02, p = .086, d = 1.13$). There were no statistically significant differences between resilient and susceptible rats in c-fos-IR in the core ($t(9) = 0.63, p = .545, d = 0.38$).

Amygdala. c-fos-IR in areas of the amygdala can be seen in Figure 5.5C. There were no statistically significant differences between resilient and susceptible rats in c-fos-IR in the central amygdala ($t(9) = -0.07, p = .944, d = 0.04$) and the basolateral amygdala ($t(9) = 0.07, p = .940, d = 0.05$).

Granular Insular Cortex. There was no statistically significant difference between resilient and susceptible rats in c-fos-IR in the granular insular cortex ($t(9) = 0.59, p = .570, d = 0.36$; Figure 5.5D)

Lateral Hypothalamus. There was no statistically significant difference between resilient and susceptible rats in c-fos-IR in the lateral hypothalamus ($t(9) = -1.34, p = .213, d = 0.81$; Figure 5.5E).

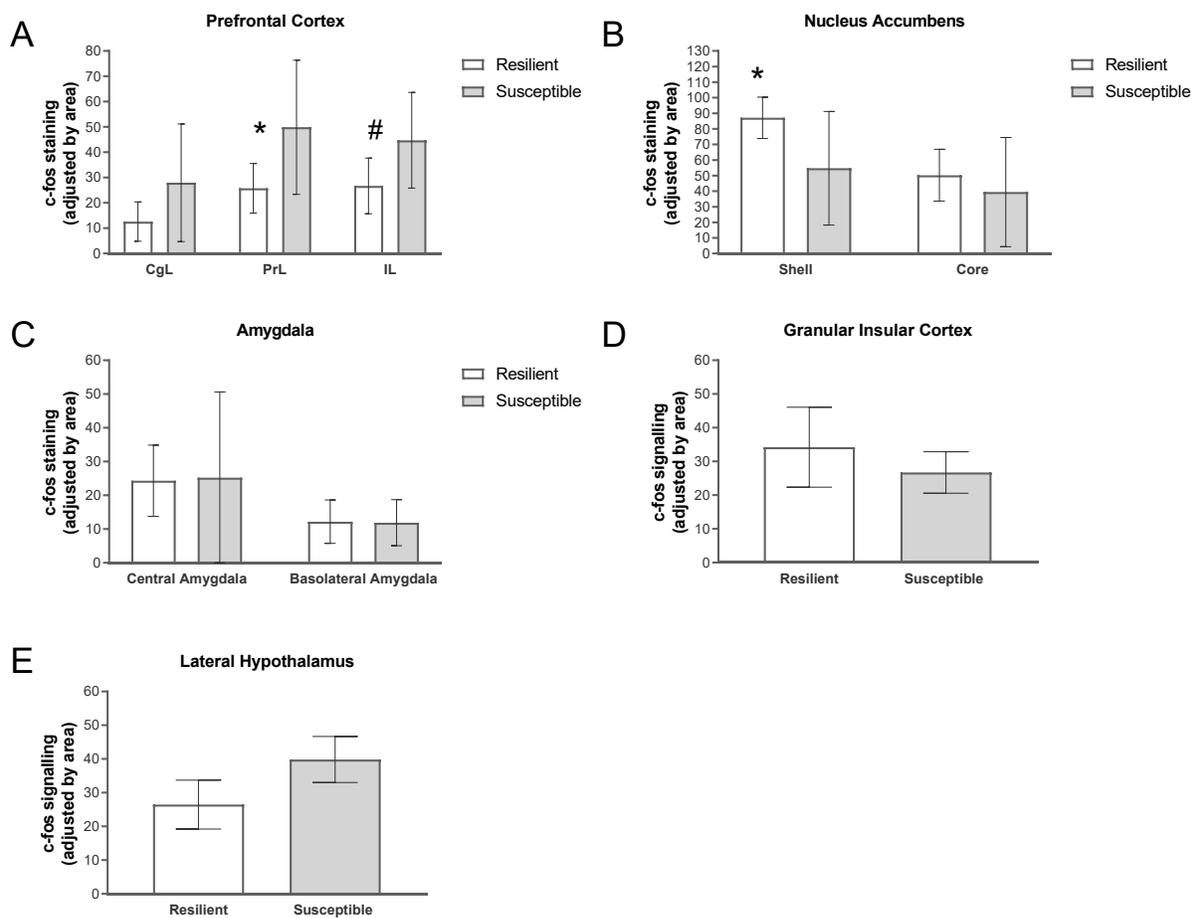


Figure 5.5. Number of fos immunoreactive (IR) cells as a measure of neural activity during restriction phase 2 in resilient rats ($n = 5$) and susceptible rats ($n = 6$). (A) c-fos-IR in different areas of the prefrontal cortex, $*p = .088$, compared to susceptible; $\#p = .093$, compared to susceptible, (B) c-fos-IR in the NAcc core and shell; $*p = .086$, (C) c-fos-IR in different areas of the amygdala, (D) c-fos-IR in the granular insular cortex, and (E) c-fos-IR in the lateral hypothalamus.

Discussion

The goal of the present experiment was to examine if there are differences in neural activity between rats that are resilient to ABA development versus those that are more susceptible. Rats were exposed to two bouts of ABA and the percentage of the initial body weight at the end of the second bout of ABA was used to identify susceptible rats. On the last day of the second bout of ABA, brains were collected during the postprandial phase and fos immunohistochemistry was used as a nonspecific measure of neuronal activity. The PFC, NAcc, hypothalamus, amygdala, and granular insular cortex were surveyed for differences in c-fos-IR between resilient and susceptible rats. We found that ABA susceptible rats showed a trend for more c-fos-IR (i.e., more neuronal activity) in the prelimbic cortex and infralimbic cortex of the PFC and lower activity in the NAcc shell compared to resilient rats, though these differences did not reach statistical significance. No differences were observed in the other sampled brain regions.

The finding of potentially increased neuronal activity in two regions of the PFC and decreased activity in the NAcc shell of ABA-susceptible rats compared to resilient rats is consistent with reports in humans with AN. Indeed, one neurobiological hypothesis explaining the extreme food restriction in AN is that powerful inhibitory influences from the PFC exert excessive cognitive control over bottom-up appetitive responses involved in the regulation of homeostasis, reward processing, and motivational drive (Foldi et al., 2017). This view is supported by imaging studies in individuals with AN that show enhanced neural activity in the dorsolateral PFC (dlPFC) leading to heightened cognitive control and reduced reward function. For instance, a functional MRI study showed that recovered AN patients not only engaged the dlPFC to a greater extent than control participants during reward anticipation, but they also failed to deactivate the dlPFC following feedback (Ehrlich et al., 2015). Presentation of visual food stimuli has also been shown to increase dlPFC activity in acute AN relative to control participants (Brooks et al., 2012). Wagner et al. (2007) also reported increased activation in the dorsal striatum and PFC in AN and Brooks et al. (2011) showed that dlPFC volume in AN seems to be positively correlated with dietary restraint. In the present experiment, we used fos immunohistochemistry as a nonspecific measure of regional neuronal activity. While our results suggest that susceptible rats had more PFC activity than resilient rats, it is impossible to

determine specifically which pathways were involved. Based on the above findings from the literature of heightened dlPFC activity and increased cognitive control in AN, further research would be warranted to determine whether enhanced PFC activity observed here is related to increased cognitive control in ABA susceptible rats.

A recent study by Milton et al. (2020) provides support for the view that inhibitory influences from the PFC may be exerting excessive cognitive control over appetitive responses in AN. Using DREADDs, the authors showed that inhibiting the activity of neurons projecting from the mPFC to the NAcc during ABA prevented weight loss and improved flexibility in a reversal learning task. These findings are consistent with our observation of higher PFC activity in ABA susceptible rats and offer one possible hypothesis as to the role of this heightened activity. Another possibility is that the increased c-fos expression observed in our ABA susceptible rats reflects increased activation of pyramidal neurons projecting from the PFC to motor centers. This is supported by research in mice that investigated whether individual differences in ABA correlate with lengths of axo-somatic contacts made by GABAergic terminals onto layer V pyramidal neurons in the mPFC (Chen, Wable, Chowdhury, & Aoki, 2016). The authors found that contact length was negatively correlated with wheel running and suggested that this may be a cellular mechanism by which excitatory input from the PFC to motor centers of the brain are more strongly inhibited in ABA resilient mice which dampens their running wheel activity and allows them to maintain their body weight compared to susceptible mice. These interesting findings offer yet another potential explanation for the increased neuronal activity observed in the PFC of our ABA susceptible rats. Our methodology, however, does not allow us to determine whether this heightened activity in susceptible rats occurs in the pathway between the PFC and motor centers of the brain or in the PFC's projections to the mesolimbic pathway involved in motivation.

To the best of our knowledge, this is the first experiment that compared brain activity across several relevant regions between ABA susceptible and resilient rats and our findings of heightened activity in the PFC of susceptible rats are consistent with human and animal research, providing further support for the use of ABA as a model of AN-like symptoms. It should be noted that fos immunohistochemistry is a time-sensitive technique that provides information about activity in the 20-90 minute window before the tissue was collected. It is possible that

tissue collection during a different time could have resulted in differences in other brain areas. In other words, absence of observable differences between resilient and susceptible rats in the other brain regions sampled does not necessarily mean that no differences exist. Furthermore, the present experiment did not include a non-ABA control group which prevents us from drawing conclusions about the effect of ABA on brain activity in these areas of interest.

EXPERIMENT 3.2. RESPONSE INHIBITION BEFORE AND AFTER ABA IN RESILIENT AND SUSCEPTIBLE RATS

Impulsivity has been defined as a predisposition toward rapid, unplanned reactions to internal or external stimuli without regard to the negative consequences of these reactions to the individual or to others (Moeller et al., 2001). This multidimensional construct involves disruptions within a wide range of cognitive processes including attention, perception, and coordination of motor or cognitive responses (Fineberg et al., 2014). Underlying these processes is a cortico-striatal neurocircuit composed of a striatal component (ventral striatum) that drives impulsive behaviour and a prefrontal component (ventromedial PFC) that exerts inhibitory control (Fineberg et al., 2014). Given our findings in the previous experiment of a trend for higher activity in areas of the PFC and lower activity in the NAcc shell in susceptible rats compared to resilient rats, we assessed for trait differences in impulsivity. The data on impulsivity in eating disorder populations to date has been inconclusive and the construct has yet to be examined in the ABA model and more specifically, relative to ABA resilience and susceptibility.

Individuals with AN are often described as over-controlled and harm-avoidant, and displaying perseverative, obsessive, and rigid thinking styles (Lilenfeld, Wonderlich, Riso, Crosby, & Mitchell, 2006). It has been proposed that this overcontrol results from excessive response inhibition that may contribute to individual's ability to severely restrict caloric intake in AN (Ehrlich et al., 2015). This view is consistent with the description of AN as a neurobiologically-based disorder with abnormal higher-order cognitive control. Indeed, this overcontrol has provided the basis for the development of Radically-Open Dialectical Behaviour Therapy for the treatment of AN which aims to reduce behavioural rigidity (Lynch et al., 2013).

Despite these clinical observations, however, neurocognitive studies specifically assessing impulsivity in AN are scarce and inconclusive. The majority of studies on impulsivity in eating disorders have focused on BN and have shown evidence for increased impulsivity in this population (Friederich, Wu, Simon, & Herzog, 2013). Only a few studies, however, have assessed impulsivity in AN and results have either shown no behavioural evidence for abnormalities (Claes, Nederkoorn, Vandereycken, Guerrieri, & Vertommen, 2006), evidence for higher impulsivity (Díaz-Marsá et al., 2008; Kane, Loxton, Staiger, & Dawe, 2004; Rosval et al.,

2006), or evidence of better performance (Butler & Montgomery, 2005; Claes et al., 2005) on measures of impulsivity. Results appear to be strongly dependent on the subtype of AN studied (restrictive vs binge-purge), the age of the participants, stimuli used, and the dimension of impulsivity being assessed (Waxman, 2009).

Response inhibition is one of the many components of the multifaceted construct of impulsivity. It represents the neurocognitive ability that allows us to inhibit or suppress prepotent, automatic responses following changes in environmental circumstances and plays an important role in goal-directed behaviour (Weinbach, Lock, & Bohon, 2020). Tasks commonly used to assess response inhibition are the go/no-go and stop-signal reaction time tasks. In the go/no-go tasks, individuals perform motor responses to “go” cues but are to refrain from responding when a “no-go” cue is presented (Fineberg et al., 2014). On the stop-signal reaction time tasks, individuals make motor responses to “go” cues, but attempt to suppress responses when a stop signal is presented after the presentation of the go cue (Fineberg et al., 2014). Only a few studies have examined response inhibition in AN (for review see Bartholdy et al., 2016). In a recent study, Weinbach et al. (2020) assessed response inhibition in adolescents with AN-restrictive type using a food-stop-signal reaction time tasks in which participants’ ability to inhibit prepotent responses following exposure to high- and low-calorie food images was measured. Their results revealed superior ability of adolescents with AN-restrictive type, compared to healthy participants, to inhibit actions following exposure to high-calorie food images while no difference was observed following exposure to low-calorie foods. The authors suggest that this stronger activation of response inhibition in AN may contribute to patients’ ability to severely restrict eating.

To date, there have been no published studies examining impulsivity in the ABA model in rodents. Behavioural paradigms designed to assess response inhibition in rodents, such as the go/no-go task akin to those used in humans, have been developed and used in different research contexts. In the present study, we used a go/no-go procedure adapted from Cooper et al. (2014) in which rats were taught to press a lever for a sucrose pellet in response to a cue light and to resist pressing the lever (response inhibition) to receive a sucrose pellet in response to a cue tone. This procedure allowed us to measure pre-cue responses as well as success rates during “go” and

“no-go” trials. It was hypothesized that ABA-susceptible rats would show less impulsivity in the form of less pre-cue responses and higher success rates compared to ABA-resilient rats.

Method

Subjects

Female Sprague Dawley rats ($n = 12$; 125-150 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec) and housed in a colony room on a 12:12 hr reverse light/dark cycle. Upon arrival, rats were initially pair-housed in plastic shoebox cages and were then separated into individual shoebox cages on day 3. After 7 days of acclimation in the animal care facility, rats were transferred to the laboratory where they were permanently housed in running wheel cages inside sound-attenuating boxes until the end of the experiment. Except for the restriction phases and go/no-go training and testing periods, rats had *ad libitum* access to both food and water. Body weight (g), food intake (g), and water intake (g) was monitored daily at ZT 11-12.

Apparatus

Running Wheel Cages. See “General Methodology” section.

Elevated Plus Maze. See “General Methodology” section.

Forced Swim Task. See “General Methodology” section.

Go/No-Go task. The Go/No-Go task was conducted in operant conditioning chambers (Coulbourn Instruments, Holliston, MA, 29.0 cm × 29.0 cm × 25.5 cm). Operant conditioning chambers were located within individual sound attenuating boxes, and each chamber contained two levers located 11.0 cm above the grid floor. A single lever (left or right, counterbalanced between animals) was used during training and testing. The chamber also contained a cue-light and an audio indicator (Sonalert, 2.9 KHz, 10-20 dB above background level) located above the active lever, and a red house-light positioned on the top of the wall opposite the levers.

Procedure

The detailed timeline is depicted in Figure 5.6.

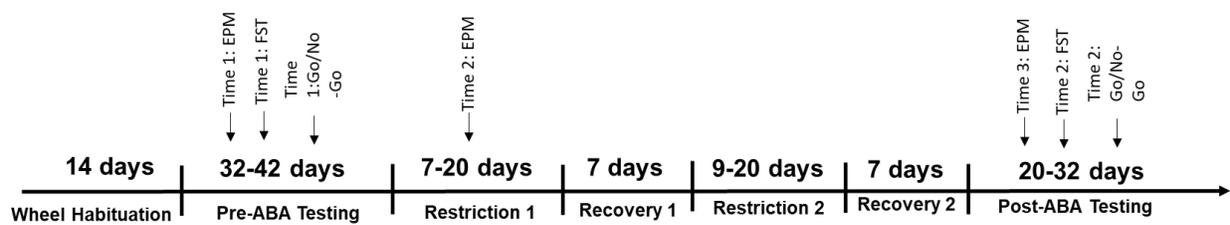


Figure 5.6. Timeline for experiment 3.2.

Wheel habituation phase. Seven days following arrival, rats were transferred from the animal care facility to the individual running wheel cages where they were permanently housed for the remainder of the experiment. During this phase, all rats had *ad libitum* food and water and continuous access to the running wheel. This phase lasted 14 days.

Pre-ABA behavioural testing phase. During this phase, rats underwent a series of behavioural tests (described below). As the time required for training during the go/no-go task varied across animals, this phase lasted from a minimum of 32 days to a maximum of 42 days.

Elevated Plus Maze Time 1. EPM testing occurred on the first or second day of the pre-ABA behavioural testing phase during a 1.5-hour time window corresponding to ZT 15-16.5, where ZT 0 is lights on. See “General Methodology” section for more details.

Forced Swim Task Time 1. FST took place between the 4th and 7th day of this phase, 2 days following EPM. The task was administered during the active phase (ZT 15-17) under regular light conditions. See “General Methodology” for the detailed procedure.

Go/No-Go Task. The day after the FST, the go/no-go task was initiated. At the start of each trial, the lever extended for a short pre-cue period during which responses on the lever were recorded but not reinforced. The house light was on during pre-cue periods.

Go Training. For Go trials, the stimulus light above a single lever (left or right, counterbalanced between animals) was illuminated and rats were trained to press the lever to obtain a sucrose pellet on a continuous reinforcement schedule. Rats had 15 s to respond on the lever after which point the lever retracted and the trial was scored as an omission. A 3 s intertrial interval (ITI) followed a lever press or trial omission. During the ITI, the house light was off and the lever was retracted. Once rats acquired two consecutive days of > 80% success in at least 100 trials/30-min, they began No-Go training. This training period ranged from 17 to 25 days.

No-Go Training. During No-Go training, rats were trained to refrain from pressing the lever in order to obtain a sucrose pellet. No-Go trials began with a tone sounding for 15 s while the stimulus light remained off and the lever was extended. Responses on the lever terminated the trial and were scored as an error and the chamber reverted to ITI conditions. If rats refrained from pressing the lever during the 15-s tone, they received a sucrose pellet. Once rats acquired

two consecutive days of > 80% success in at least 100 trials/30-min, they began Go/No-Go testing. This training period ranged from 4 to 6 days.

Go/No-go Testing. Rats underwent 4 days of testing with 120 daily trials in which Go and No-Go trials varied randomly at a 25:75 ratio. Trials began with the extension of the lever and house light turning on initiating a variable pre-cue period (9-24 s). High pre-cue responses are interpreted as impulsive behaviour. Following the pre-cue period, the stimulus light or tone was presented and the trial continued as described above. Following this series of behavioural tests, rats were given two days undisturbed (with the exception of daily weighing) to recover before starting ABA.

Restriction Phase 1. Following the 2-day recovery, the first restriction phase began. Food was removed at ZT 13. On the following days, rats had access to food for 1 hr/day between ZT 12-13. The restriction phase was terminated when a rat reached the starvation criterion (weight loss of 25% of initial body weight) or following 3 consecutive days without weight loss for a minimum of 7 days of restriction and a maximum of 20 days.

Elevated Plus Maze Time 2. On the 4th or 5th day of restriction phase 1, rats were again tested in the EPM using the above procedure.

Recovery Phase 1. Following restriction phase 1, rats recovered for 7 days. During this recovery period, rats had *ad libitum* access to food and water with continued access to the running wheels.

Restriction Phase 2. Following the 7-day recovery, the second restriction phase began using the same procedure as in restriction phase 1.

Recovery Phase 2. Rats were again allowed to recover from the restriction phase for 7 days during which they had *ad libitum* access to food and water with continued access to the running wheels.

Post-ABA behavioural testing phase. During this phase, rats underwent the same behavioural testing procedures as pre-ABA behavioural testing. The same procedures for the EPM, FST, and Go/No-Go Task described above were employed here.

Statistical Analysis

A median split on percent of initial body weight after 6 days of the restriction phase 1 was used to retrospectively assign rats to the resilient or susceptible groups whereby rats with the lowest percent body weight (i.e., those that lost the most weight during ABA) were considered susceptible. These two traits were then used as categorical independent variables in a series of ANOVAs.

To characterize the development of ABA, three 2 x 2 mixed ANOVAs were used for the following three dependent variables: running wheel activity, food intake, and change in body weight. *Trait* (resilient vs. susceptible) was used as the between-subjects factor while *phase* (average during last 6 days of the wheel habituation vs. average during the first 6 days of the restriction phase 1) was used as the within-subjects factor.

To compare the two bouts of ABA, three 2 x 2 mixed ANOVAs were used for the following three dependent variables: running wheel activity, food intake, and change in body weight. *Trait* (resilient vs. susceptible) was used as the between-subjects factor while *phase* (average during first 7 days of restriction phase 1 vs. average during first 7 days of restriction phase 2) was used as the within-subjects factor.

Two 2 x 3 mixed ANOVAs were used to analyze proportion of time spent in open arms and total closed arm entries in the EPM. *Trait* (resilient vs. susceptible) was used as the between-subjects factor while *phase* (pre-ABA, restriction phase 1, and post-ABA) was used as the within-subjects factor.

Three 2 x 2 mixed ANOVAs were used to analyze immobility time, latency to immobility, and time spent climbing in the FST. *Trait* (resilient vs. susceptible) was used as the between-subjects factor while *phase* (pre-ABA vs. post-ABA) was used as the within-subjects factor.

A series of 2 x 2 mixed ANOVAs were used to analyse Go/No-Go task results. *Trait* (resilient vs. susceptible) was used as the between-subjects factor while *phase* (pre-ABA vs. post-ABA) was used as the within-subjects factor. A separate ANOVA was conducted for each of the following independent variables: 1) days to criterion: number of days required to complete the Go and No-Go trainings; 2) Pre-cue responses: number of lever presses during the variable

pre-cue period averaged across the 4 testing days; 3) Success rate in Go trials: percentage of success rate during Go trials averaged across 4 testing days; 4) Success rate in No-Go trials: percentage of success rate during No-Go trials averaged across 4 testing days.

Results

Data Integrity

A total of 12 rats were used in this experiment. One rat showed unprecedented low running wheel activity throughout the experiment and was considered to be an outlier and removed from analyses. This resulted in 5 rats in the resilient group and 6 rats in the susceptible group.

Development of ABA

Running wheel activity. As seen in Figure 5.7A, food restriction resulted in a statistically significant increase in running wheel activity whereby average daily wheel rotations was higher during the restriction phase 1 compared to activity during the running wheel habituation phase (*phase*: $F(1, 9) = 46.59, p < .001, \eta_p^2 = 0.84$). Susceptible rats had higher running wheel activity compared to the resilient rats overall (*trait*: $F(1, 9) = 8.85, p = .016, \eta_p^2 = 0.50$). Importantly, the increase in running wheel activity from the habituation phase to the restriction phase was more pronounced for the susceptible rats compared to the resilient rats, as indicated by the statistically significant *phase x trait* interaction ($F(1, 9) = 11.80, p = .007, \eta_p^2 = 0.57$).

Food intake. As expected, rats ate significantly less during the restriction phase compared to the habituation phase (*phase*: $F(1, 9) = 302.26, p < .001, \eta_p^2 = 0.97$; Figure 5.7B). There was no difference in food intake between traits and no interactions (*trait*: $F(1, 9) = 0.03, p = .859, \eta_p^2 < .01$; *phase x trait*: $F(1, 9) = 0.15, p = .710, \eta_p^2 = 0.02$).

Change in body weight. As seen in Figure 5.7C, rats lost significantly more weight during the restriction phase compared to the habituation phase (*phase*: $F(1, 9) = 410.50, p < .001, \eta_p^2 = 0.98$). There was no difference in change in body weight between traits and no interactions (*trait*: $F(1, 9) = 0.21, p = .658, \eta_p^2 = .02$; *phase x trait*: $F(1, 9) = 1.21, p = .299, \eta_p^2 = 0.12$). During restriction phase 1, susceptible rats took an average of 9 days to reach starvation

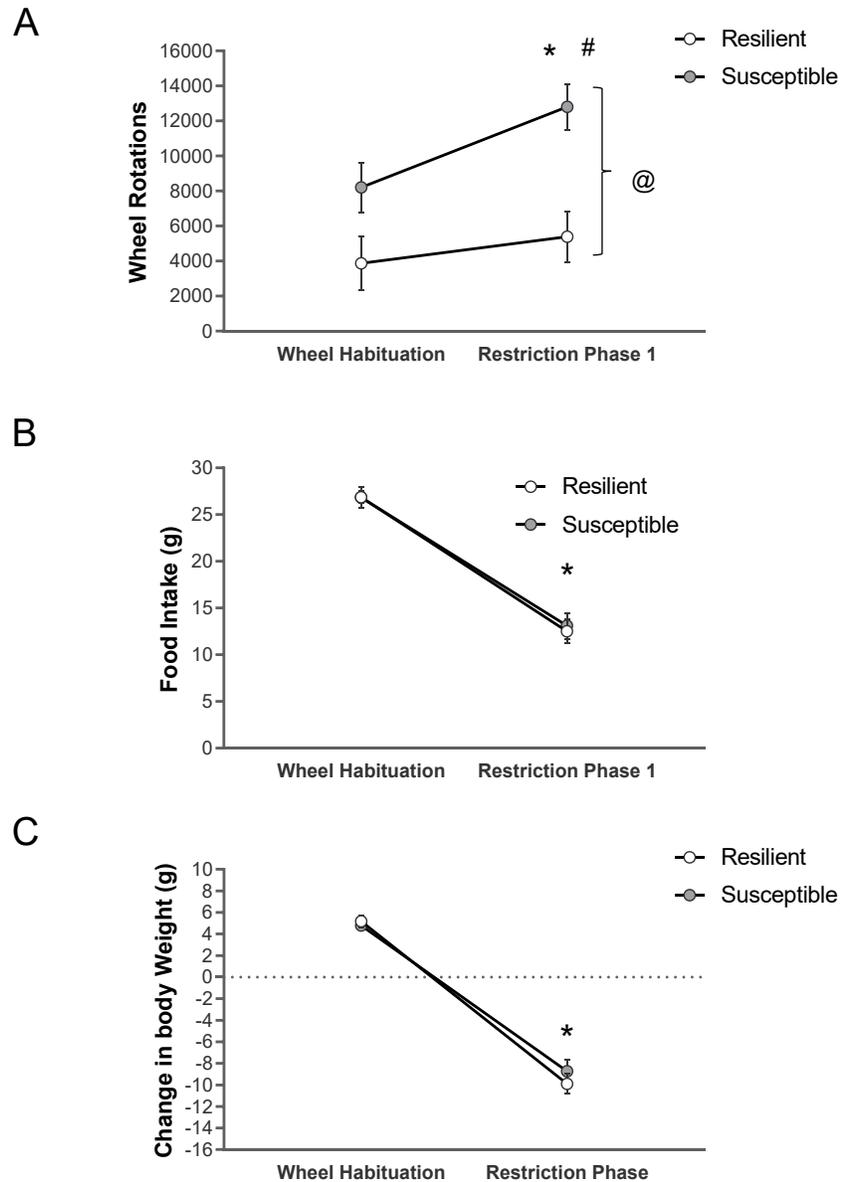


Figure 5.7. The effect of ABA on running wheel activity, food intake, and in body weight in resilient rats ($n = 5$) and susceptible rats ($n = 6$). (A) Mean running wheel activity during the habituation phase and restriction phase 1. * $p < .001$, main effect of *phase*; # $p = .016$, main effect of *trait*; @ $p = .007$, *phase x trait* interaction. (B) Mean food intake during the habituation phase and restriction phase 1. * $p < .001$, main effect of *phase*. (C) Mean change in body weight during the habituation phase and restriction phase 1. * $p < .001$, main effect of *phase*.

criterion while only 1 rat in the resilient group reached starvation criterion (after 15 days). The remaining 4 rats in the resilient condition reached stability criteria.

Comparison between bout 1 and bout 2 of ABA

Running wheel activity. Running wheel activity during both restriction phases can be seen in Figure 5.8A. Running wheel activity was significantly lower during the second restriction phase compared to the first (*phase*: $F(1, 9) = 7.72, p = .021, \eta_p^2 = 0.46$). Susceptible rats ran significantly more than resilient rats overall (*trait*: $F(1, 9) = 10.98, p = .009, \eta_p^2 = 0.55$). Importantly, the decrease in running wheel activity from the first to the second restriction phase was apparent in susceptible rats only (*phase x trait*: $F(1, 9) = 12.91, p = .006, \eta_p^2 = 0.59$).

Food intake. Food intake during both restriction phases can be seen in Figure 5.8B. Food intake was significantly higher during the second restriction phase compared to the first (*phase*: $F(1, 9) = 27.67, p = .001, \eta_p^2 = 0.76$). There was no difference in food intake between traits and no interactions (*trait*: $F(1, 9) = 0.53, p = .485, \eta_p^2 = 0.06$; *phase x trait*: $F(1, 9) = 1.59, p = .240, \eta_p^2 = 0.15$).

Change in body weight. Change in body weight during both restriction phases can be seen in Figure 5.8C. Rats appeared to lose less weight during the second restriction phase compared to the first, though this difference did not reach statistical significance (*phase*: $F(1, 9) = 4.29, p = .068, \eta_p^2 = 0.32$). There was no difference in change in body weight between traits and no interactions (*trait*: $F(1, 9) = 1.88, p = .204, \eta_p^2 = 0.17$; *phase x trait*: $F(1, 9) = 0.07, p = .803, \eta_p^2 = 0.01$).

Behavioural testing

Elevated Plus Maze. As seen in Figure 5.9A, proportion of time spent in open arms of the maze did not significantly differ across trait or experimental phases and there was no interaction (*phase*: $F(2, 18) = 2.32, p = .127, \eta_p^2 = 0.21$; *trait*: $F(1, 9) = 0.20, p = .667, \eta_p^2 = 0.02$; *phase x trait*: $F(2, 18) = 0.68, p = .518, \eta_p^2 = 0.07$). Closed arm entries did not significantly differ across trait or experimental phases and there was no interaction (*phase*: $F(2, 18) = 0.08, p = .920, \eta_p^2 = 0.01$; *trait*: $F(1, 9) = 0.01, p = .990, \eta_p^2 < 0.01$; *phase x trait*: $F(2, 18) = 0.40, p = .679, \eta_p^2 = 0.04$; Figure 5.9B).

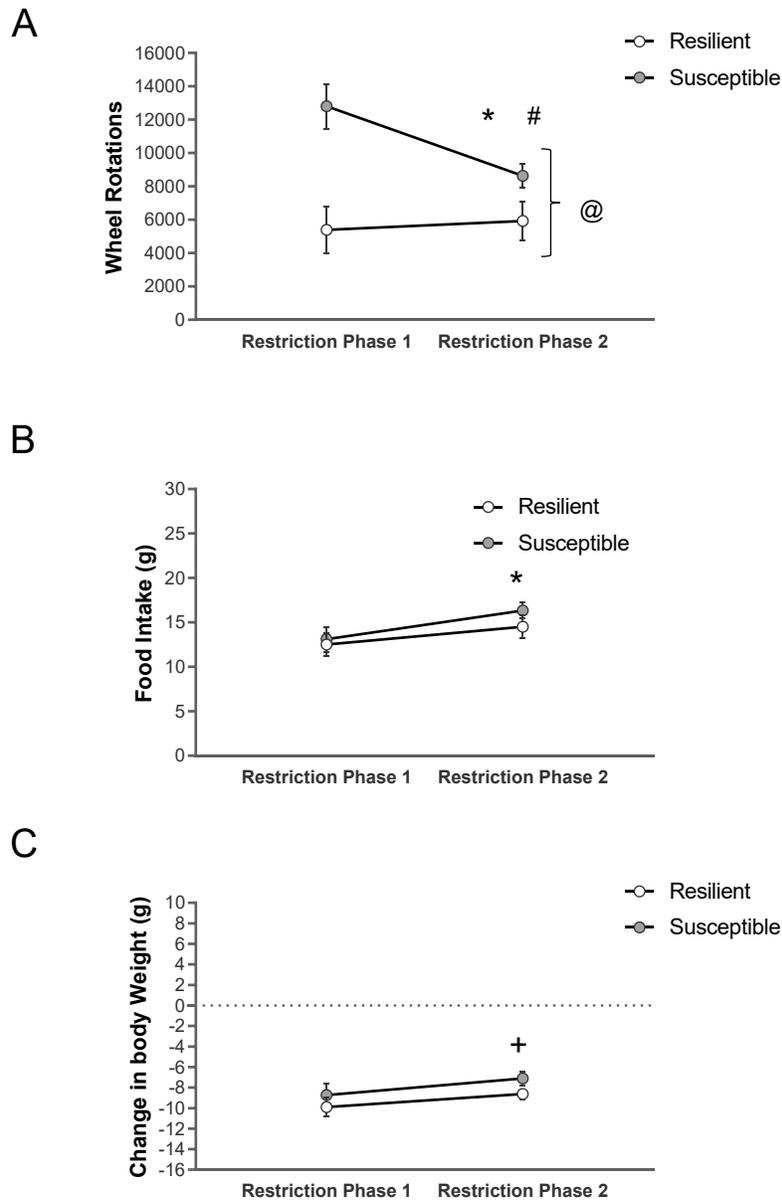


Figure 5.8. Differences in running wheel activity, food intake, and body weight in resilient rats ($n = 5$) and susceptible rats ($n = 6$) across two bouts of ABA. (A) Mean running wheel activity during the restriction phase 1 and restriction phase 2. * $p = .021$, main effect of *phase*; # $p = .009$, main effect of *trait*; @ $p = .006$, *phase x trait* interaction. (B) Mean food intake during the restriction phase 1 and restriction phase 2. * $p = .001$, main effect of *phase*. (C) Mean change in body weight during the restriction phase 1 and restriction phase 2. + $p = .068$, main effect of *phase*.

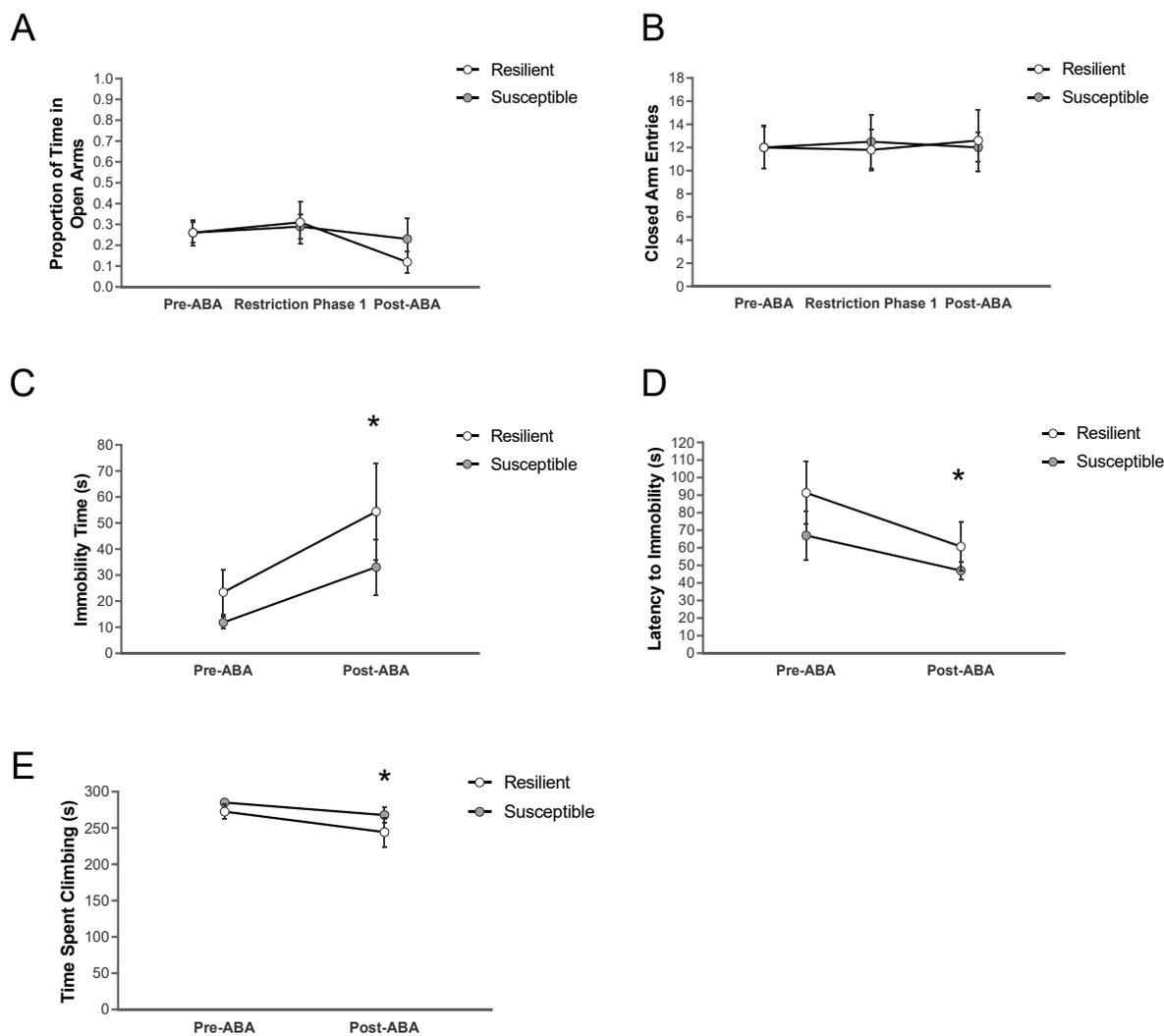


Figure 5.9. Behaviours in the Elevated Plus Maze and the Forced Swim Test in resilient rats ($n = 5$) and susceptible rats ($n = 6$) across different experimental phases. (A) Proportion of time spent in the open arms in the EPM test during pre-ABA, restriction phase 1, and post-ABA. (B) Closed arm entries in the EPM test during pre-ABA, restriction phase 1, and post-ABA. (C) Total immobility time in the FST during pre-ABA and post-ABA phases. * $p = .013$, main effect of *phase*. (D) Latency to immobility in the FST during pre-ABA and post-ABA phases. * $p = .002$, main effect of *phase*. (E) Time spent climbing in the FST during pre-ABA and post-ABA phases. * $p = .029$, main effect of *phase*.

Forced Swim Test. As seen in Figure 5.9C, there was a statistically significant increase in immobility time from pre-ABA to post-ABA (*phase*: $F(1, 9) = 9.55, p = .013, \eta_p^2 = 0.52$). There were no differences between resilient and susceptible rats and no significant interaction (*trait*: $F(1, 9) = 1.58, p = .240, \eta_p^2 = 0.15$; *phase x trait*: $F(1, 9) = 0.34, p = .577, \eta_p^2 = 0.04$). Latency to immobility significantly decreased from pre-ABA to post-ABA (*phase*: $F(1, 9) = 17.48, p = .002, \eta_p^2 = 0.66$) with no differences between resilient and susceptible rats and no significant interaction (*trait*: $F(1, 9) = 1.93, p = .303, \eta_p^2 = 0.12$; *phase x trait*: $F(1, 9) = 0.75, p = .409, \eta_p^2 = 0.08$, Figure 5.9D). Time spent climbing significantly decreased from pre-ABA to post-ABA (*phase*: $F(1, 9) = 6.72, p = .029, \eta_p^2 = 0.43$) with no differences between resilient and susceptible rats and no significant interaction (*trait*: $F(1, 9) = 1.58, p = .240, \eta_p^2 = 0.15$; *phase x trait*: $F(1, 9) = 0.34, p = .545, \eta_p^2 = 0.04$, Figure 5.9E).

Go/No-Go Task.

Days to criterion. Rats required less days to reach criterion post-ABA compared to pre-ABA (*phase*: $F(1, 9) = 200.26, p < .001, \eta_p^2 = 0.32$; Figure 5.10A). There were no differences between resilient and susceptible rats and no interaction (*trait*: $F(1, 9) = 1.46, p = .258, \eta_p^2 = 0.14$; *phase x trait*: $F(1, 9) = 0.57, p = .471, \eta_p^2 = 0.06$).

Pre-cue responses. As seen in Figure 5.10B, pre-cue responses decreased significantly from pre-ABA to post-ABA (*phase*: $F(1, 9) = 22.07, p = .001, \eta_p^2 = 0.71$). There were no differences between resilient and susceptible rats and no interaction (*trait*: $F(1, 9) = 1.12, p = .317, \eta_p^2 = 0.11$; *phase x trait*: $F(1, 9) = 0.02, p = .892, \eta_p^2 < 0.01$).

Success rate in go trials. Success rate in “go” trials did not differ between resilient and susceptible rats or across experimental phases and there was no interaction (*phase*: $F(1, 9) = 0.08, p = .785, \eta_p^2 = 0.01$; *trait*: $F(1, 9) = 0.27, p = .619, \eta_p^2 = 0.03$; *phase x trait*: $F(1, 9) = 0.40, p = .544, \eta_p^2 = 0.04$; Figure 5.10C).

Success rate in no-go trials. As seen in Figure 5.10D, success rate in “no-go” trials significantly increased from pre-ABA to post-ABA (*phase*: $F(1, 9) = 15.58, p = .003, \eta_p^2 = 0.63$). There were no differences between resilient and susceptible rats and no interaction (*trait*: $F(1, 9) < .01, p = .977, \eta_p^2 < 0.1$; *phase x trait*: $F(1, 9) = 0.30, p = .600, \eta_p^2 = 0.03$).

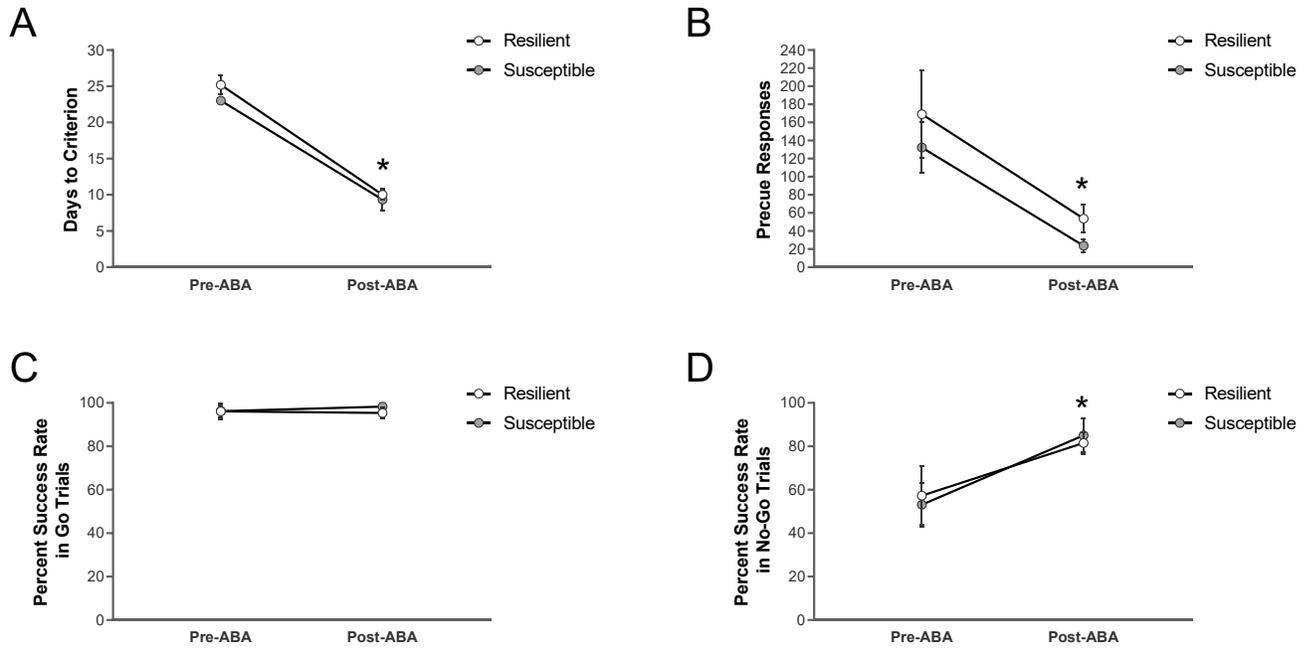


Figure 5.10. Performance in the Go/No-Go task pre- and post-ABA in resilient rats ($n = 5$) and susceptible rats ($n = 6$). (A) Days to criterion. * $p < .001$, main effect of *phase*. (B) Precue responses. * $p = .001$, main effect of *phase*. (C) Percent success rates in “go” trials. (D) Percent success rates in “no-go” trials. * $p = .003$, main effect of *phase*.

Discussion

The main goal of the present experiment was to examine if differences exist between resilient and susceptible rats on a measure of response impulsivity following two bouts of ABA. This is the first experiment assessing impulsivity in ABA. Given our previous findings that susceptible rats appeared to have higher activity in areas of the PFC compared to resilient rats and based on observations of overcontrol in humans with AN, it was hypothesized that susceptible rats would show less impulsivity via superior response inhibition abilities compared to resilient rats. Anxiety- and depression-like behaviours were also assessed using the EPM and FST, respectively.

Individual differences in response to ABA were diminished

As in all our previous experiments, food restriction resulted in increased running wheel activity and this increase was more pronounced in susceptible rats compared to resilient rats. Surprisingly, however, resilient and susceptible rats did not differ in terms of weight loss in response to the food restriction suggesting that the individual differences were less robust than in previous experiments. It should be noted, however, that the analysis included the first 6 days of the restriction phase only as no data was available for the most susceptible rats after this time. While ABA may have been slower to develop in the present experiment, we did find that all the susceptible rats eventually reached starvation criterion while 4 out of the 5 resilient rats never met this cut off – instead, they reached stability criteria of no weight loss for 3 consecutive days.

The ABA effect was more robust during the first bout of ABA compared to the second

We found that rats appeared to adjust their behaviour during the second bout of ABA compared to the first, thereby reducing the gap between resilient and susceptible rats. More specifically, susceptible rats decreased their running wheel activity from the first to second bout, all rats increased their food intake and appeared to lose less weight during the second ABA compared to the first. These results are somewhat inconsistent with reports that two bouts of ABA in C57/BL6 mice exacerbates individual differences allowing for the study of resilience and susceptibility (Chowdhury et al., 2013). Similar to our findings, these authors do report prolonged survival during a second bout of ABA suggesting behavioural adaptation, but the distinction between resilient and susceptible animals is accentuated as opposed to the reduction

of trait differences observed in the present experiment. Our findings are consistent with our own results from experiments 2.3 and 3.1 in which a two-bout protocol was used and also resulted in diminished ABA response during the second bout. We had initially posited that surgery and the presence of minipumps in experiment 2.3 had interfered with ABA behaviour during the second bout in experiment 2.3. Given that rats in the present experiment were not exposed to any surgical procedure, this explanation now appears improbable. Instead, age is a more likely explanation and is discussed in the General Discussion under “methodological considerations”.

There were no trait differences in anxiety-like behaviours in the EPM

In the present experiment, rats were tested on the EPM before ABA, during the first bout of ABA, and following two bouts of ABA and a period of recovery. We found that there were no differences in anxiety-like behaviours between resilient and susceptible rats and at the different phases of testing. This was the first experiment to examine anxiety in resilient and susceptible rats during and after ABA. We had previously tested rats on the EPM prior to ABA (experiment 2.1) and had failed to observe differences between resilient and susceptible rats. Our current results are consistent with these previous findings that ABA-susceptible rats do not show increased baseline anxiety-like behaviours compared to resilient rats. Our findings, however, contrast those using the ABA model in mice whereby anxiety-like behaviour in the EPM during ABA has been positively correlated with running wheel activity (i.e., susceptibility; Wable, Min, & Aoki, 2015) and the experience of two bouts of ABA during adolescence resulted in anxiety-like behaviours on the EPM in adulthood (Kinzig & Hargrave, 2010; Lee & Kinzig, 2017). These results are further discussed in the General Discussion.

Depression-like behaviours in the FST increased after two bouts of ABA

Depression-like behaviours were measured using the FST before ABA and following two bouts of ABA and a period of recovery. Unlike our previous findings (experiment 2.1) of reduced immobility time and increased latency to immobility in susceptible rats compared to resilient rats prior to ABA, we did not, in the present experiment, observe any differences between resilient and susceptible rats before or after ABA. We did, however, find that rats showed longer immobility time after the two bouts of ABA compared to pre-ABA. While the interpretation of behaviour in the FST is controversial (see General Discussion), the classical interpretation would suggest that the increased immobility time observed in our rats is a display

of learned helplessness suggesting that the experience of two bouts of ABA increases depression-like behaviour. Of course, this interpretation should be considered with caution as no non-ABA control group was used here to rule out other possible explanations. The observation of lasting effects of ABA on depression-like behaviour is consistent with what is reported in humans with AN whereby depressive symptoms do not remit following weight restoration (Meehan, Loeb, Roberto, & Attia, 2006)

There were no differences between resilient and susceptible rats in response inhibition

The main goal of experiment 3.2 was to assess response inhibition, a component of impulsivity, in rats exposed to ABA. To the best of our knowledge, this is the first experiment of its kind. Rats were trained and tested in the go/no-go task prior to ABA and were again re-tested post-ABA allowing us to examine whether response inhibition changed after two bouts of ABA and whether susceptible rats show enhanced response inhibition compared to resilient rats. We found no differences in response inhibition before and after ABA nor between resilient and susceptible rats. Given that this was the first experiment examining impulsivity in ABA, no comparisons can be made. Interpretation is also complicated by the fact that no control group was used. This design was chosen to specifically assess for differences between resilient and susceptible rats after our previous findings of heightened activity in the PFC of susceptible rats. In retrospect, however, it would have been ideal to include a non-ABA control group to first examine the effect of ABA on response inhibition. Interpretation of our results is also limited by the subdued individual differences in response to ABA compared to our previous experiments. It is therefore possible that differences between resilient and susceptible rats may have been observed in a cohort with clearer individual differences. We have repeatedly found that ABA is more robust in rats during a first bout of ABA compared to a second. Future studies examining response inhibition may benefit from testing the rats after one bout of ABA. It would also be interesting to assess response inhibition during exposure to ABA though the use of sucrose as a reward may interfere with ABA development. There is a possibility that our go/no-go task was not challenging enough resulting in what appears to have been a ceiling effect in success rates in both “go” and “no-go” trials. More complex procedures may be more sensitive to any potential differences in impulsivity between resilient and susceptible rats that may not have been captured here. Finally, we cannot ignore the possibility that there are simply no differences in response

inhibition between resilient and susceptible rats. This conclusion may be premature, however, until more studies are carried out.

CHAPTER 5 SUMMARY

The goal of the experiments presented in chapter 5 was to further investigate ABA susceptibility by examining neuronal activity and impulsive behaviours.

In *experiment 3.1*, c-Fos immunohistochemistry was used to assess for differences in neuronal activity between resilient and susceptible rats after experiencing two bouts of ABA. Brain regions were selected based on their involvement in hunger, feeding, homeostasis, reward processing, and cognitive functioning and included the PFC, NAcc, hypothalamus, amygdala and the granular insular cortex. We found that susceptible rats showed a statistical trend for more neuronal activity in the prelimbic cortex and infralimbic cortex of the PFC and lower activity in the NAcc shell compared to resilient rats. These results, though not statistically significant, were in line with studies in AN indicating that enhanced neural activity in the PFC might lead to heightened cognitive control and reduced reward functioning.

In *experiment 3.2*, we built on these findings by comparing impulsivity, a neurocognitive construct reliant on proper PFC functioning, in resilient and susceptible rats. More specifically, response inhibition was assessed using a Go/No-Go task following two bouts of ABA. Depression- and anxiety-like behaviours were also assessed using the FST and EPM, respectively, before and after ABA. No differences were observed between resilient and susceptible rats, and pre- and post-ABA on the EPM or the Go/No-Go task. Immobility time in the FST was higher after two bouts of ABA compared to before, though no trait differences were observed. This suggests that the experience of ABA may increase depression-like behaviours though the absence of a non-ABA control condition limits this interpretation. Importantly, and as in previous experiments, rats adjusted their behaviour in a second bout of ABA thereby diminishing the individual differences in ABA response and may partially explain why we failed to observe significant differences in neural activity, FST, and the Go/No-Go task between resilient and susceptible rats.

**CHAPTER 6: COCAINE SELF-ADMINISTRATION IN ABA AND ITS RELATION TO
ABA SUSCEPTIBILITY**

ABSTRACT

The prevalence rate of substance abuse has been reported to be 5 times higher in populations with eating disorders compared with the general population. The most abused substances in individuals with anorexia nervosa (AN) and bulimia nervosa are amphetamines, cocaine, and marijuana. The elevated rates of substance abuse in AN corroborate neuroimaging studies demonstrating both functional and structural abnormalities in areas of the brain known to be involved in reward processing. While reward processing and underlying brain circuits have begun to be investigated in the activity-based anorexia (ABA) model of AN-like symptoms, no studies have been published on drug-taking and drug-seeking behaviours in rodents exposed to ABA. The overarching goal of this study was therefore to investigate cocaine-taking behaviours in ABA and, more specifically, to determine whether differences exist between ABA-resilient and susceptible rats in cocaine self-administration. After acclimation to the running wheel, rats were divided into the ABA and sedentary conditions. Rats in the ABA condition experienced two bouts of ABA (food available for 60 min/day and continuous wheel access). Each bout of ABA was followed by a 7-day recovery. Rats in the sedentary condition experienced the same food restrictions but had locked wheels. Following recovery from the ABA phase, the cocaine phase began for all rats and consisted of 12 days of cocaine self-administration training, self-administration testing, 15 days of withdrawal, 10 days of restriction, and a cue-induced reinstatement test. We found that rats with a history of ABA, compared to sedentary rats, were more motivated to work for cocaine and were more resistant to extinction. Interestingly, resilient rats showed a trend for higher motivation and slower extinction compared to susceptible rats, though interpretation of these findings are limited by small sample sizes. Overall, our results suggest that the combination of severe food restriction and excessive physical activity results in alterations in reward processing that persist even after recovery from ABA. Possible explanations for these results are explored here and in the general discussion (Chapter 7).

EXPERIMENT 4. INTRODUCTION

According to the National Center on Addiction and Substance Use at Columbia University (2003), the prevalence of substance abuse is five times higher in populations with eating disorders, compared with the general population. Though the research on substance abuse in AN specifically is scarce, there is evidence to suggest that the higher prevalence of substance abuse in AN may contribute to the exceptionally high mortality rate of 5-20% in AN (Fichter, Quadflieg, & Hedlund, 2006; Steinhausen, 2002). In addition to studies on substance use and abuse in AN, neuroimaging studies in AN have demonstrated both functional and structural abnormalities in areas of the brain known to be involved in reward processing. While researchers have begun to examine neurocircuitry involved in reward processing in AN, no studies have been published on drug-taking and drug-seeking behaviours in rodents exposed to AN. The main goal of the present study was to begin examining whether AN-resilient versus AN-susceptible rats would show different cocaine self-administration and cocaine-seeking behaviours. A secondary goal was to assess the effects of a history of AN on later cocaine self-administration and cocaine seeking in adult female rats.

It is well documented that AN is the psychiatric illness with this highest mortality rate. Keel et al. (2003) studied mortality in eating disorders and found that severity of substance use positively predicted both death and time-to-death in individuals with AN demonstrating the importance of furthering our understanding of this comorbidity. The risk of developing an eating disorder is reportedly associated with illicit drug use and abuse, and, specifically, self-reported cocaine use was found to be higher in at-risk women (9%) than in not at-risk women (5%; Gadalla & Piran, 2007). While it was previously believed that substance abuse was more prevalent in BN and less common in AN, more recent research has demonstrated that this is not the case when the different subtypes of AN are considered: AN-restrictive type (AN-R) and AN-binge/purge type (AN-BP). In an international sample of individuals with AN (including AN-R and AN-BP), 26% of participants self-reported lifetime substance use and 14% reported lifetime substance abuse including cocaine (16%; Root et al., 2010). Prevalence of substance use disorders differed across AN subtypes with higher prevalence in the AN-BP group compared to the AN-R group (Root et al., 2010). Consistent with this, Herzog, Nussbaum, and Marmor, (1996) reported that prevalence rates of substance use disorders did not differ between AN and BN but were generally higher in

individuals who purged (BN and AN-BP) compared to individuals who did not purge (AN-R). They reported a lifetime prevalence rate of substance use disorders of 17% and found that the most commonly abused substances were AMPH, cocaine, and marijuana. While it is speculated that the appetite-suppressing effects of AMPH and cocaine may partly explain why individuals with ED are more likely to use and abuse these drugs, it is also hypothesized that abnormalities in reward circuitry may be at play.

Individuals with AN have been described as anhedonic and ascetic (Kaye et al., 2013). Harrison, O'Brien, Lopez, and Treasure (2010) found that, compared to healthy control participants, individuals with AN possess an increased ability to delay reward. The authors also found that individuals with AN have high punishment sensitivity and low reward reactivity during both the acute and recovered states of AN (Harrison et al., 2010). As anhedonia is dependent on the mesolimbic DA pathway, many researchers have focused on examining this pathway in AN, finding evidence of dysfunction. For instance, reductions in CSF DA metabolites has been observed in malnourished individuals with AN and shown to persist after recovery (Kaye et al., 1999). Individuals who have recovered from AN have also been shown to have increased binding of DA D2 and D3 receptors in the ventral striatum (Montague, Hyman, & Cohen, 2004; Schultz, 2004) which may be a response to a reduction in extracellular DA. Altered frequency of functional polymorphisms of DA D2 receptor genes have also been reported in AN and may impact receptor transcription and translation efficiency (Bergen et al., 2005).

While no studies have yet been published on drug-taking behaviours in ABA, several studies have begun to explore reward processing and circuitry in ABA and have provided evidence for disruptions in reward processing and circuitry. For instance, the ABA phenotype has been shown to be ameliorated by increasing the hedonic value of food using a high-fat diet suggesting that ABA may be associated with reward deficiency (Brown, Avena, & Hoebel, 2008). As previously discussed in Chapter 4 (see experiment 2.3 introduction) many studies have succeeded in preventing the development of ABA by targeting DA signalling using selective and nonselective DA antagonists providing evidence for DA dysregulation in ABA (e.g., Hillebrand, Van Elburg, Kas, Van Engeland, & Adan, 2005; Klenotich, Ho, McMurray, Server, & Dulawa, 2015; Routtenberg & Kuznesof, 1967; Verhagen, Luijendijk, Korte-Bouws, Korte, & Adan, 2009). In more recent years, optogenetics and chemogenetic technologies have made it possible

to identify and manipulate pathway-specific projections. Using a dual viral strategy, Foldi, Milton, and Oldfield (2017) demonstrated that direct activation of the VTA-NAcc reward pathway prevented ABA via increased food intake and survival time.

Taken together, these findings provide support for reward deficiency in individuals with AN. The reward deficiency hypothesis suggests that substance use is the consequence of a low functioning mesolimbic DA system which predisposes an individual to seek psychoactive substances and behaviours to release DA in the reward circuit to overcome DA deficits (Blum et al., 2000). According to this hypothesis, it is possible that reward deficits in AN may be linked to increased prevalence rate of substance use disorders in this population. While results from studies using the ABA model seem consistent with clinical studies suggesting reward deficiency, no studies have examined drug-taking behaviours in ABA. Doing so would not only begin to answer the question of whether or not ABA susceptibility is associated with higher or lower drug taking and seeking, but it would also provide a protocol by which drug use could be examined in an animal model of AN-like behaviours. We therefore sought to, for the first time, use well-known animal models of drug taking, seeking, and relapse in rats with a history of ABA.

Drug self-administration procedures provide a means for studying addiction-like behaviours under controlled conditions. In rodents, the animals are trained to self-administer a drug of abuse by performing an operant task, such as lever-pressing or nose-poking, to obtain a pre-determined dose of the drug that is paired with a discrete cue (e.g., tone or light). After animals have reliably learned to self-administer the drug, behavioural economics concepts can be used to determine how much they are willing to “pay” for the drug (Newman & Ferrario, 2020). Briefly, this can be done through the study of the “demand curve” which represents the consumption of the drug as a function of the price paid (i.e., work performed to obtain it) which informs us about the animal’s motivation to obtain a drug. Forced abstinence and cue-induced reinstatement can then be used to model relapse. Cue-induced reinstatement is one of the most commonly used animal models of relapse as it has both face and predictive validity (Epstein, Preston, Stewart, & Shaham, 2006; Shalev, Grimm, & Shaham, 2002). In this procedure, once rats have established stable drug self-administration, the drug is removed and the behaviour is extinguished. Extinction is reached when the animal demonstrates a low level of responding.

Next, a trigger is used to elicit renewed drug seeking (i.e., responses on the previously drug-associated lever or nose poke). The increase in non-reinforced responding on the lever (or nose poke) is the operational measure for the relapse of drug seeking. Despite its extensive use and face and predictive validity (Epstein et al., 2006; Shalev et al., 2002), the reinstatement model has been criticized as the extinction procedure does not mimic the human condition of abstinence. To address this weakness, we also included a period of forced withdrawal immediately following self-administration training and testing and prior to the reinstatement procedure. During forced withdrawal, rats are returned to their home cage for varying periods of time (here 15 days) during which the drug is not available.

This is the first study to examine drug-taking behaviours in ABA. Using the procedures described above, we sought to examine how drug-taking and seeking behaviours relate to individual differences in ABA susceptibility. Our design also allowed us to ask if a history of ABA is associated with more drug-taking behaviours. Based on the high prevalence of substance use disorders in AN as well as the evidence of altered reward processing in AN and ABA, we hypothesized that rats with a history of ABA, compared to sedentary rats, would show more motivation to work for cocaine, resistance to extinction, and higher cue-induced reinstatement. More importantly, we expected that these results would be amplified in ABA-susceptible rats compared to ABA-resilient rats.

Method

Subjects

A total of 30 Sprague Dawley female rats (125-150 g; Charles River, Saint-Constant, Quebec) were used for this experiment. As in previous experiments, rats were kept on a 12:12 hr reverse light/dark cycle and their body weight, food intake, and water intake was monitored daily at ZT 11-12 throughout the experiments. Upon arrival, rats were individually housed in a colony room and allowed to acclimate for 7 days before being relocated into running wheel cages. With the exception of the restriction phases, rats had *ad libitum* access to both food and water throughout the experiment.

Surgical Procedure

Intravenous catheterization took place during the second recovery phase (described in the procedure section below). Catheters were constructed out of silastic tubing (Inner Diameter: 0.51 mm, Outer Diameter: 0.94 mm; Dow Corning, Midland, MI, USA) that were cut to 12 cm. Rats were anesthetized with 2% isoflurane for the duration of the surgery. Once fully anesthetized, the lower neck, chest, and head were shaved and disinfected with soap and 70% ethanol. Pre-surgical care included subcutaneous injections of 2 ml of saline (0.9%) to maintain hydration, penicillin (450,000 IU/rat) to reduce the risk of infection, and Atropine (0.1 mg/kg) to prevent mucus buildup during surgery. A small incision was made on the skull and another was made on the neck to expose the jugular vein. Once the jugular vein was isolated, a small incision was made and approximately 3 cm of the catheter was inserted into the vein and secured in place with 3 silk sutures. The other end of the catheter was then subcutaneously threaded to the incision on the skull and was attached to a modified 22-gauge cannula (Plastics One, Roanoke, VA, USA). Following the surgery until the withdrawal phase, catheters were flushed daily with heparin and gentamicin in sterile saline (7.5IU + 12.0 µg per day per rat) to prevent catheter blockage and infection.

Apparatus

Running wheel cages. See “General Methodology” section.

Operant chambers. Operant conditioning chambers (Med Associates Inc., St. Albans, VT, USA, 31.8 cm × 25.4 cm × 21.0 cm) were used during cocaine self-administration, testing, extinction, and reinstatement. Operant conditioning chambers were located within individual sound attenuating boxes. Each chamber was equipped with two retractable levers located 5.0 cm above the grid floor. One lever was the designated “active” drug-paired lever and the other was the “inactive” non-drug paired lever (side was counterbalanced between rats). A white cue-light and tone indicator (Sonalert, 2.9 KHz, 10-20 dB above background level) were located above the active lever, and a red house-light was positioned on the top of the wall opposite the levers. A Tygon tubing (Inner Diameter: 0.50 mm, Outer Diameter: 1.52 mm; Saint-Gobain Performance Plastics, Granville, NY, USA), shielded by a metal spring, connected the rats’ catheters to a swivel (Instech Laboratories, Plymouth Meeting, PA, USA or Lomir Biomedical Inc., Quebec, Canada) located at the top of the chamber. The swivel was attached to a 20-ml syringe via Tygon

tubing. The syringe was mounted onto a pump outside the chamber. MED-PC software was used to run all programs in this experiment.

Drugs

Cocaine (diacetylmorphine HCl; National Institute on Drug Abuse, Baltimore, Maryland, USA) was dissolved in sterile saline and delivered at a dose of 0.10 mg/kg/infusion. All other compounds used for intracranial injections are detailed in their respective chapter methodology.

Procedure

ABA Phase. A timeline of the entire experiment can be seen in Figure 6.1.

Running wheel habituation phase. After 7 acclimation days, all rats were re-located into running wheel cages and allowed to habituate for 19 days. All rats had unrestricted access to running wheels, food, and water. See the general method section for more details.

Restriction Phases. Matched on running wheel activity, rats were assigned into experimental and control groups: ABA ($n = 12$) and sedentary ($n = 12$). For the remainder of the experiment, ABA rats had unrestricted access to running wheels, whereas sedentary rats had locked running wheels. During the restriction phases, all rats were food restricted for 23 hrs per day. The first restriction phase began with the removal of food 1 hr following the onset of the dark phase. On subsequent days, rats had access to food for 1 hr immediately at the onset of the dark phase. The first restriction phase was terminated at a 25% body weight loss or a maximum duration of 7 days. A 7-day recovery followed with unrestricted access to food and water. A second restriction phase began for a maximum duration of 9 days, followed by a 7-day recovery.

Cocaine phase. All manipulations in this phase of the study began at the onset of the dark cycle, and rats were returned to their running wheel cages until the following day when the procedure was complete. All rats had unrestricted access to food during this phase.

Self-administration training. Following one acclimation day, rats received daily 4-hr training sessions for 12 days. All behavioural sessions began at the onset of the dark cycle. The start of the session was marked by the extraction of the retractable ‘active’ lever, illumination of the house light and cue light, as well as a tone (2.9 kHz; 10 dB above background level). Pressing the active lever resulted in a 0.5 mg/kg infusion of cocaine over 12 s, which turned the

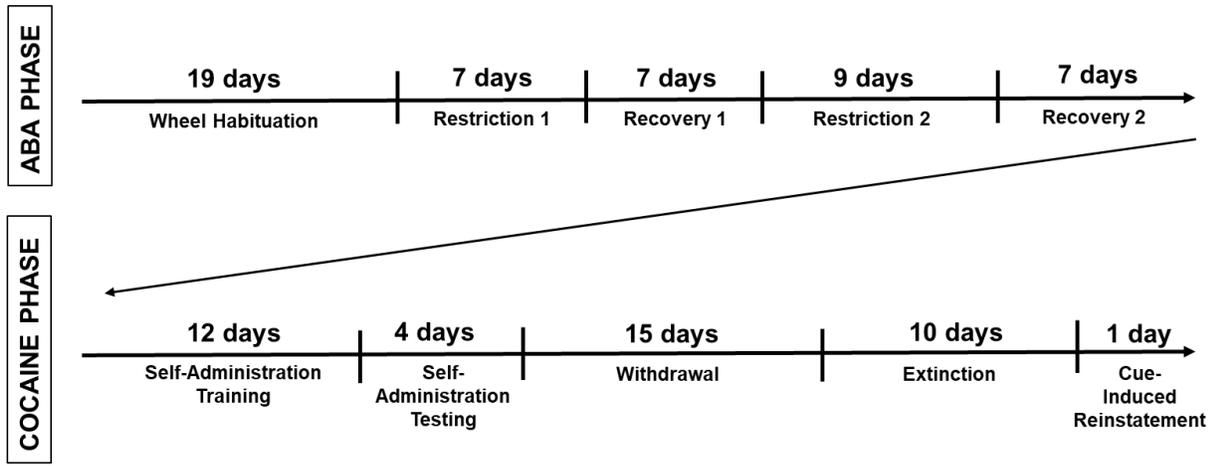


Figure 6.1. Timeline for experiment 4.

house light off, activated the light/tone cue, and initiated a 20-s time-out period during which additional responses on the active lever had no consequences. Termination of the time-out was marked by the light/tone cue turning off and the re-activation of the house light.

Self-administration testing. Rats were repeatedly tested over 4 days, with daily eleven 10-min sessions. The start of the session was only marked by the extraction of the active lever and the illumination of the house light. In each 10-min session, a different dose of cocaine was administered in descending order: 1120, 630, 350, 200, 110, 60, 30, 20, 10, 5, and 3 ug/kg/infusion. Pressing the active lever resulted in an infusion of cocaine, which turned the house light off and initiated a time-out period for the duration of the pump activation.

Withdrawal. Rats were returned to their running wheel cages for 15 days. Again, ABA rats had unrestricted access to running wheels, whereas sedentary rats had locked running wheels. Both groups had with unrestricted access to food and water.

Extinction. Rats received daily 4-hr sessions over 10 days. The start of the session was marked by the extraction of the retractable ‘active’ lever and the illumination of the house light, but not the cue light or tone. Pressing the ‘active’ lever had no consequences (i.e., no infusion of cocaine or cue presentation).

Cue-induced reinstatement. Rats were tested in a single 4-hr session. Reinstatement was identical to self-administration training, with the exception that pressing the ‘active’ lever resulted in the presentation of the cocaine-associated cues (light/tone) without cocaine infusion.

Statistical Analyses

A median split based on weight loss (percentage of initial body weight) on the last day of the first restriction phase was used to divide ABA rats into further resilient and susceptible groups.

Development of ABA. ANOVAs were used to analyse changes in running wheel activity, food intake, and body weight from the habituation phase to the restriction phase 1. The habituation phase represented the average of the dependent variable of interest across the last 4 days of habituation before the start of restriction phase 1. The restriction phase 1 was represented by the average of the dependent variable of interest during the first 4 days of this restriction

phase. For both food intake and body weight, two 2 x 2 mixed ANOVAs were conducted. The first used *activity condition* (sedentary vs. ABA) as the between-subject variable and *phase* (habituation phase vs. restriction phase 1) as the within-subject variable. The second ANOVA was conducted in rats in the ABA condition only and used *trait* (resilient vs. susceptible) as the between-subject variable and *phase* (habituation phase vs. restriction phase 1) as the within-subject variable. As no running wheel activity data were available for sedentary rats, a single 2 x 2 mixed ANOVA was used with rats in the ABA condition to characterize differences in activity between resilient and susceptible rats.

First bout vs. second bout of restriction. To compare running wheel activity of resilient and susceptible rats during the second bout of ABA, a 2 x 2 mixed ANOVA was conducted with *trait* (resilient vs. susceptible) as the between-subject factor and *phase* (habituation phase vs. restriction phase 2) as the within-subject factor.

For both food intake and body weight, two 2 x 2 mixed ANOVAs were conducted. The first used *activity condition* (sedentary vs. ABA) as the between-subject variable and *phase* (restriction phase 1 vs. restriction phase 2) as the within-subject variable. The second ANOVA was conducted in rats in the ABA condition only and used *trait* (resilient vs. susceptible) as the between-subject variable and *phase* (restriction phase 1 vs. restriction phase 2) as the within-subject variable.

Cocaine self-administration training. Two 2 x 12 mixed ANOVAs were conducted for each of the following dependent variables: cocaine infusions, active lever presses, inactive lever presses. For the first ANOVA, *activity condition* (sedentary vs. ABA) was the between-subject factor and *days* was the within-subject factor. The second ANOVA analysed the data of rats in the ABA condition only and used *trait* (resilient vs. susceptible) as the between-subject factor and *days* as the within-subject factor.

Self-administration test. Doses were converted into a “unit-price”, which reflected the total active lever presses required to maintain a 1-mg dose of cocaine. Each rat’s performance was then assessed graphically by plotting total responses per session as a function of unit-price. The unit-price with the highest response rate (P_{Max}) was identified and combined across all test days to produce an averaged P_{Max} for each rat (Greenwald & Hursh, 2006). A consumption curve representing the expected consumption level (Q) as a function of price was used to calculate Q_0

for each rat which represented the preferred consumption level when price was negligible. A total of four independent samples t-tests were used to compare P_{Max} and Q_0 between sedentary and ABA rats and resilient and susceptible rats.

Extinction of cocaine seeking. Two 2 x 10 mixed ANOVAs were conducted for active and inactive lever presses. For the first ANOVA, *activity condition* (sedentary vs. ABA) was the between-subject factor and *sessions* (10 extinction sessions) was the within-subject factor. The second ANOVA analysed the data of rats in the ABA condition only and used *trait* (resilient vs. susceptible) as the between-subject factor and *sessions* as the within-subject factor.

Cue-induced reinstatement of cocaine seeking. Two 2 x 2 ANOVAs were used to analyse both the active and inactive lever presses. In both ANOVAs, *session* was used as a within-subject factor and compared lever presses during the cue-induced reinstatement session to the averaged lever presses across the final three extinction sessions. In the first ANOVA, *activity condition* (sedentary vs. ABA) was used at the between-subject factor. The second ANOVA was used for rats in the ABA condition only and used *trait* (resilient vs. susceptible rats) as the between-subject factor.

Results

Data Integrity

A total of 30 rats underwent surgery. Out of these initial 30 rats, 5 rats were removed as they either did not survive the surgery or were unable to recover post-surgery. Of the remaining 25 rats that were used in the ABA phase of the experiment, an additional rat was removed as it did not acquire cocaine self-administration, likely due to a blocked catheter. This left a total of 12 sedentary rats and 12 ABA rats (5 resilient, 7 susceptible) that were used in the analyses of the ABA phase and self-administration training. During the 4 days of self-administration testing, 2 additional rats were removed due to a lack of responding during the test sessions which could have impacted subsequent extinction and cue-induced reinstatement. As such, a total of 11 sedentary rats and 11 ABA rats (4 resilient, 7 susceptible) were used in the analyses of self-administration testing, extinction, and cue-induced reinstatement.

Development of ABA

Running wheel activity.

Resilient vs. Susceptible Rats. Running wheel activity for rats in the ABA condition is displayed in Figure 6.2A. Food restriction resulted in a statistically significant increase in running wheel activity from the habituation phase to the restriction phase (*phase*: $F(1, 10) = 21.38, p = .001, \eta_p^2 = 0.68$). Susceptible rats ran significantly more than resilient rats across both the habituation and restriction phases (*trait*: $F(1, 10) = 7.07, p = .024, \eta_p^2 = 0.41$). There was no statistically significant interaction (*phase x trait*: $F(1, 10) = 0.13, p = .133, \eta_p^2 = 0.01$).

Food intake.

Sedentary vs. ABA Rats. As depicted in figure 6.2B, all rats, regardless of activity condition, ate significantly less during the restriction phase compared to the habituation phase (*phase*: $F(1, 22) = 391.18, p < .001, \eta_p^2 = 0.95$). There was no statistically significant difference in food intake between sedentary and ABA rats and no statistically significant interaction (*activity condition*: $F(1, 22) = 0.01, p = .919, \eta_p^2 < .01$; *phase x activity condition*: $F(1, 22) = 0.67, p = .423, \eta_p^2 = .03$).

Resilient vs. Susceptible Rats. A more detailed examination of rats in the ABA condition (Figure 6.3C) revealed that, while food intake was lower during the restriction phase compared to the habituation phase across all rats, there was no statistically significant difference between resilient and susceptible rats and no interaction (*phase*: $F(1, 10) = 253.00, p < .001, \eta_p^2 = 0.96$; *trait*: $F(1, 10) = 0.02, p = .893, \eta_p^2 < 0.01$; *phase x trait*: $F(1, 10) = 0.12, p = .739, \eta_p^2 = 0.01$).

Body weight.

Sedentary vs. ABA Rats. As expected, all rats lost significantly more weight during the restriction phase compared to the habituation phase and ABA rats lost significantly more weight than sedentary rats (Figure 6.2D). Importantly, a statistically significant *phase x activity condition* revealed that ABA rats lost more weight than sedentary rats during the restriction phase (*phase*: $F(1, 22) = 418.42, p < .001, \eta_p^2 = 0.95$; *activity condition*: $F(1, 22) = 15.88, p = .001, \eta_p^2 = 0.42$; *phase x activity condition*: $F(1, 22) = 8.23, p = .009, \eta_p^2 = 0.27$).

Resilient vs. Susceptible Rats. Rats in the ABA condition (Figure 6.2E) lost significantly more weight during the restriction phase compared to the habituation phase and susceptible rats

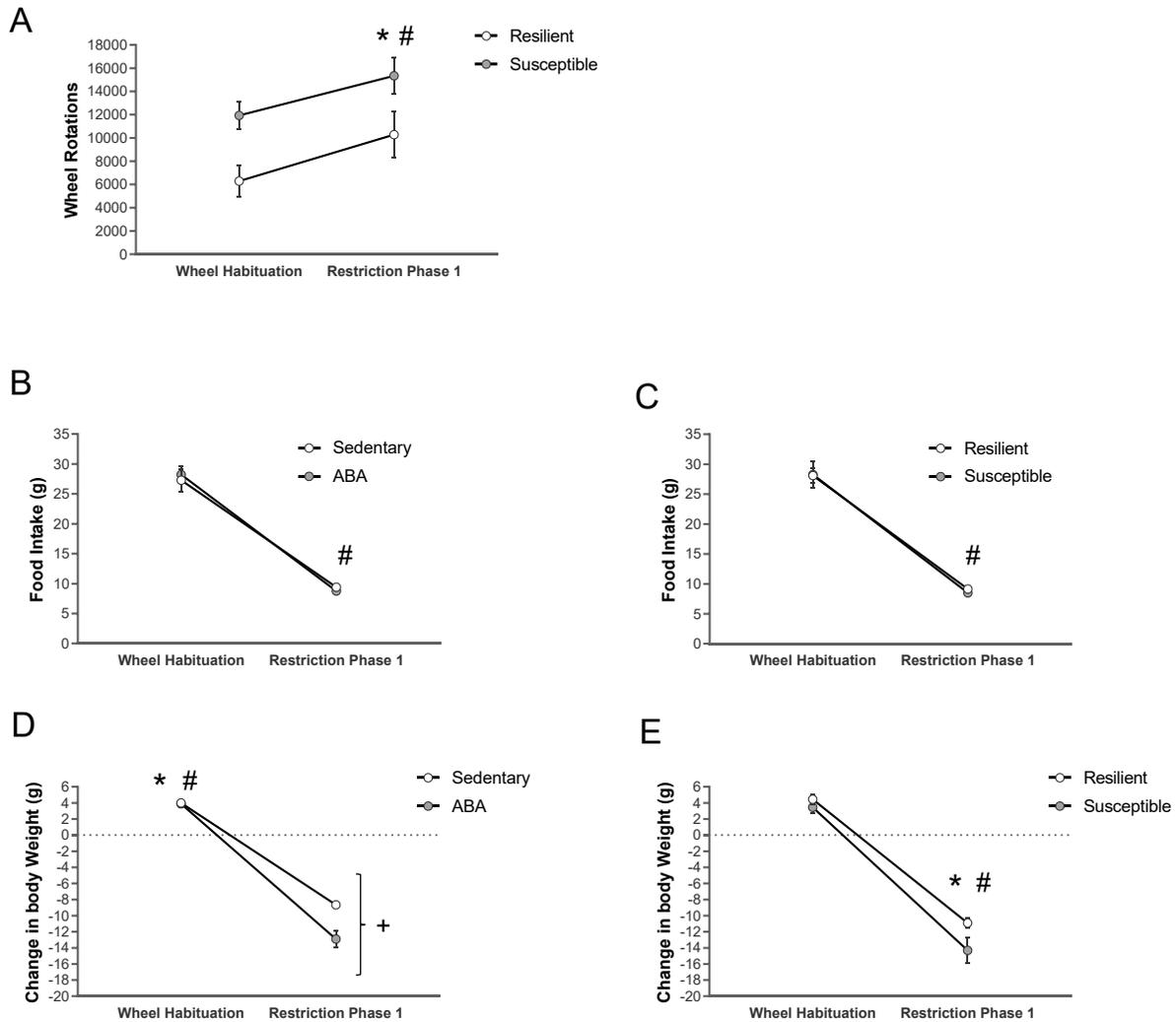


Figure 6.2. The effect of food restriction on running wheel activity, food intake, and change in body weight in sedentary ($n = 12$) versus ABA ($n = 12$) rats and in ABA-resilient ($n = 5$) versus ABA-susceptible rats ($n = 7$). (A) Mean running wheel activity during the habituation phase and restriction phase 1 in resilient and susceptible rats. $*p = .024$, main effect of *trait*; $\#p = .001$, main effect of *phase*. (B) Mean food intake during the habituation phase and restriction phase 1 in sedentary and ABA rats. $\#p < .001$, main effect of *phase*. (C) Mean food intake during the habituation phase and restriction phase 1 in resilient and susceptible rats. $\#p < .001$, main effect of *phase*. (D) Mean change in body weight during the habituation phase and restriction phase 1 in sedentary and ABA rats. $*p < .001$, main effect of *activity condition*; $\#p < .001$, main effect of *phase*; $+p = .0099$, *phase x activity condition* interaction. (E) Mean change in body weight during the habituation phase and restriction phase 1 in resilient and susceptible rats. $*p = .024$, main effect of *activity condition*; $\#p < .001$, main effect of *phase*.

lost more weight than resilient rats (*phase*: $F(1, 10) = 170.79, p < .001, \eta_p^2 = 0.95$; *trait*: $F(1, 10) = 7.01, p = .024, \eta_p^2 = 0.41$). There was no statistically significant interaction (*phase x trait*: $F(1, 10) = 0.92, p = .359, \eta_p^2 = 0.08$).

Restriction phase 1 vs restriction phase 2

Running wheel activity. Running wheel activity for rats in the ABA condition during restriction phase 1 and 2 can be seen in Figure 6.3A. Running wheel activity was significantly lower during the second restriction phase compared to the first (*phase*: $F(1, 10) = 5.35, p = .041, \eta_p^2 = 0.33$). Susceptible rats ran significantly more than resilient rats overall (*trait*: $F(1, 10) = 4.57, p = .056, \eta_p^2 = 0.29$). The interaction was not statistically significant (*phase x trait*: $F(1, 10) = 0.54, p = .478, \eta_p^2 = 0.05$).

Food intake. Food intake for sedentary and ABA rats during restriction phases 1 and 2 is depicted in Figure 6.3B. Overall, rats ate significantly more during restriction phase 2 compared to restriction phase 1 (*phase*: $F(1, 22) = 6.76, p = .016, \eta_p^2 = 0.24$). While there was no statistically significant difference between overall food intake between activity conditions (*activity condition*: $F(1, 22) = 0.38, p = .545, \eta_p^2 = 0.02$), the statistically significant *phase x activity condition* indicated that rats in the ABA condition increased their food intake during the second restriction phase while rats in the sedentary condition ate comparable amounts during both restriction phases (*phase x activity condition*: $F(1, 22) = 8.49, p = .008, \eta_p^2 = 0.28$). Across traits, all rats in the ABA condition ate more during the second restriction phase compared to the first (*phase*: $F(1, 10) = 19.17, p = .001, \eta_p^2 = 0.66$). There was no statistically significant difference in overall food intake between resilient and susceptible rats (*trait*: $F(1, 10) = 0.40, p = .541, \eta_p^2 = 0.04$), and no statistically significant interaction (*phase x trait*: $F(1, 10) = 0.15, p = .711, \eta_p^2 = 0.01$ Figure 6.3C).

Body weight. Change in body weight for sedentary and ABA rats during restriction phases 1 and 2 is depicted in Figure 6.3D. Across both activity conditions, rats lost less weight during the second restriction phase compared to the first. While the ABA rats did lose more weight overall compared to the sedentary rats, this was the case during the first restriction phase but not during the second (*phase*: $F(1, 22) = 24.06, p < .001, \eta_p^2 = 0.52$; *activity condition*: $F(1, 22) = 15.11, p = .001, \eta_p^2 = 0.41$; *phase x activity condition*: $F(1, 22) = 7.08, p = .014, \eta_p^2 =$

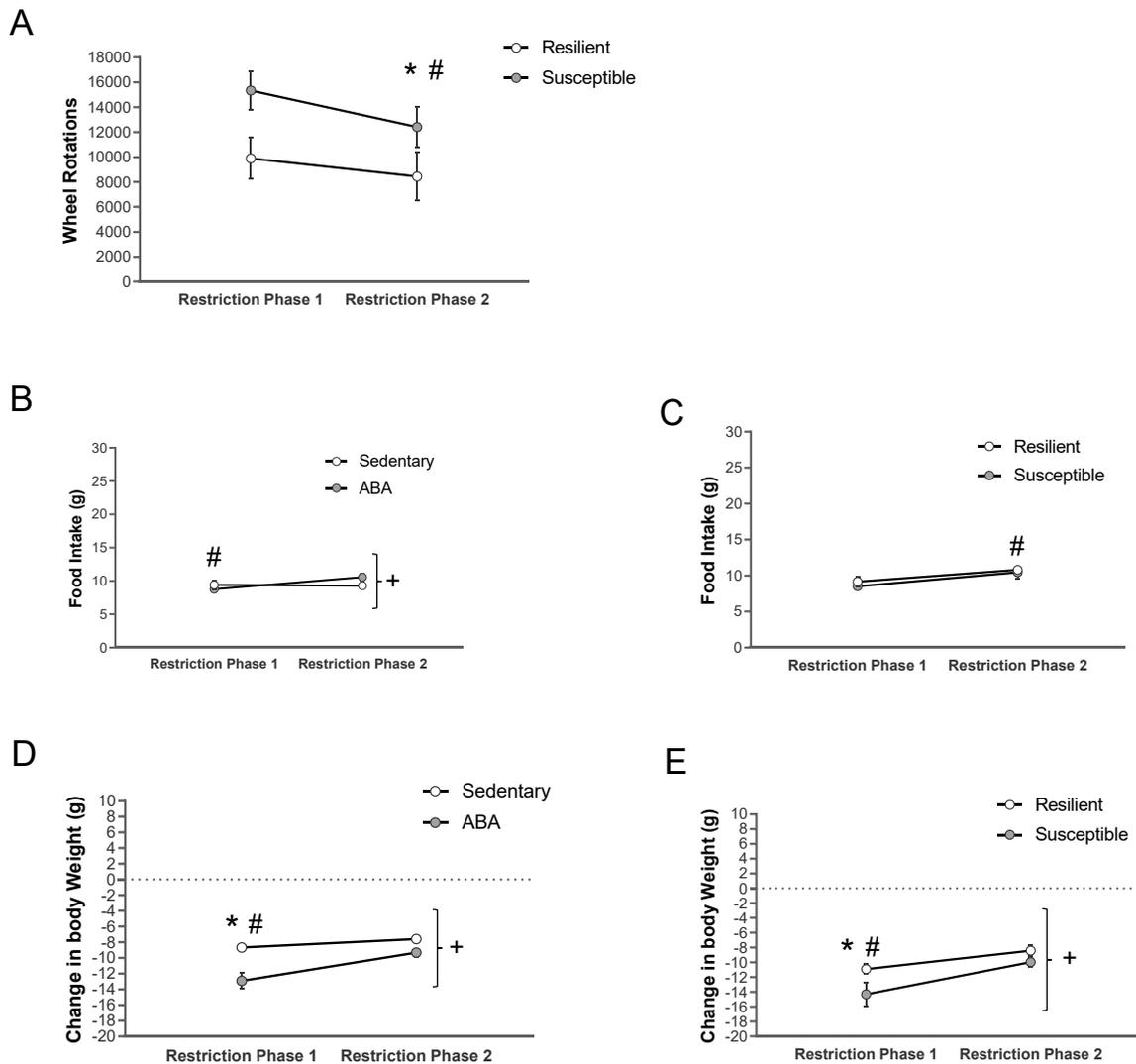


Figure 6.3. Differences in running wheel activity, food intake, and body weight in sedentary ($n = 12$) versus ABA ($n = 12$) rats and in ABA-resilient ($n = 5$) versus ABA-susceptible rats ($n = 7$). (A) Mean running wheel activity during the restriction phase 1 and restriction phase 2 in resilient and susceptible rats. $*p = .056$, main effect of *trait*; $\#p = .041$, main effect of *phase*. (B) Mean food intake during the restriction phase 1 and restriction phase 2 in sedentary and ABA rats. $\#p = .016$, main effect of *phase*; $+p = .008$, *phase x activity condition* interaction. (C) Mean food intake during the restriction phase 1 and restriction phase 2 in resilient and susceptible rats. $\#p < .001$, main effect of *phase*. (D) Mean change in body weight during the restriction phase 1 and restriction phase 2 in sedentary and ABA rats. $*p < .001$, main effect of *activity condition*; $\#p < .001$, main effect of *phase*; $+p = .014$, *phase x activity condition* interaction. (E) Mean change in body weight during the restriction phase 1 and restriction phase 2 in resilient and susceptible rats. $*p = .078$, trending effect of *trait*; $\#p = .002$, main effect of *phase*; $+p = .014$.

0.24). Across traits, all rats lost significantly less weight during the second restriction phase compared to the first and susceptible rats showed a trend for higher weight loss compared to resilient rats across both restriction phases (*phase*: $F(1, 10) = 17.42, p = .002, \eta_p^2 = 0.64$; *trait*: $F(1, 10) = 3.86, p = .078, \eta_p^2 = 0.28$; *phase x trait*: $F(1, 10) = 1.28, p = .285, \eta_p^2 = 0.11$; Figure 6.3E).

Relationships between ABA and Cocaine Self-Administration

Self-administration training.

Sedentary vs. ABA rats. Active lever presses, inactive lever presses, and infusions for sedentary and ABA rats can be seen in Figure 6.4A-B. All rats quickly learned to distinguish between the active and inactive levers across self-administration training days, acquiring reliable cocaine self-administration. There were no differences between sedentary and ABA rats in overall number of infusions, active, and inactive lever presses. On the final day of training under a FI-20 s schedule of reinforcement, the mean (\pm SEM) numbers of cocaine infusions, active, and inactive lever presses for sedentary rats were 84.67 (11.29), 99.75 (15.14), and 2.92 (1.08), respectively. For rats in the ABA condition, number of cocaine infusions, active, and inactive lever presses were 82.75 (6.62), 97.50 (9.98), and 2.50 (0.73), respectively. See Table 6.1 for detailed statistics.

Resilient vs. susceptible rats. Active lever presses, inactive lever presses, and infusions for resilient and susceptible rats can be seen in Figure 6.5A-B. A more detailed examination of rats in the ABA condition indicated that there were no differences between resilient and susceptible rats in overall number of infusions, active, and inactive lever presses. On the final day of training under a FI-20 s schedule of reinforcement, the mean (\pm SEM) numbers of cocaine infusions, active, and inactive lever presses for resilient rats were 75.00 (11.69), 86.00 (9.02), and 1.40 (0.68), respectively. For susceptible rats, number of cocaine infusions, active, and inactive lever presses were 88.29 (7.77), 105.71 (15.72), and 3.29 (1.11), respectively. See Table 6.2 for detailed statistics.

Self-administration test.

Sedentary vs. ABA rats. Across self-administration test sessions, averaged P_{Max} was significantly higher in ABA rats compared to sedentary rats, $t(20) = -2.13, p = .046, d = 0.91$

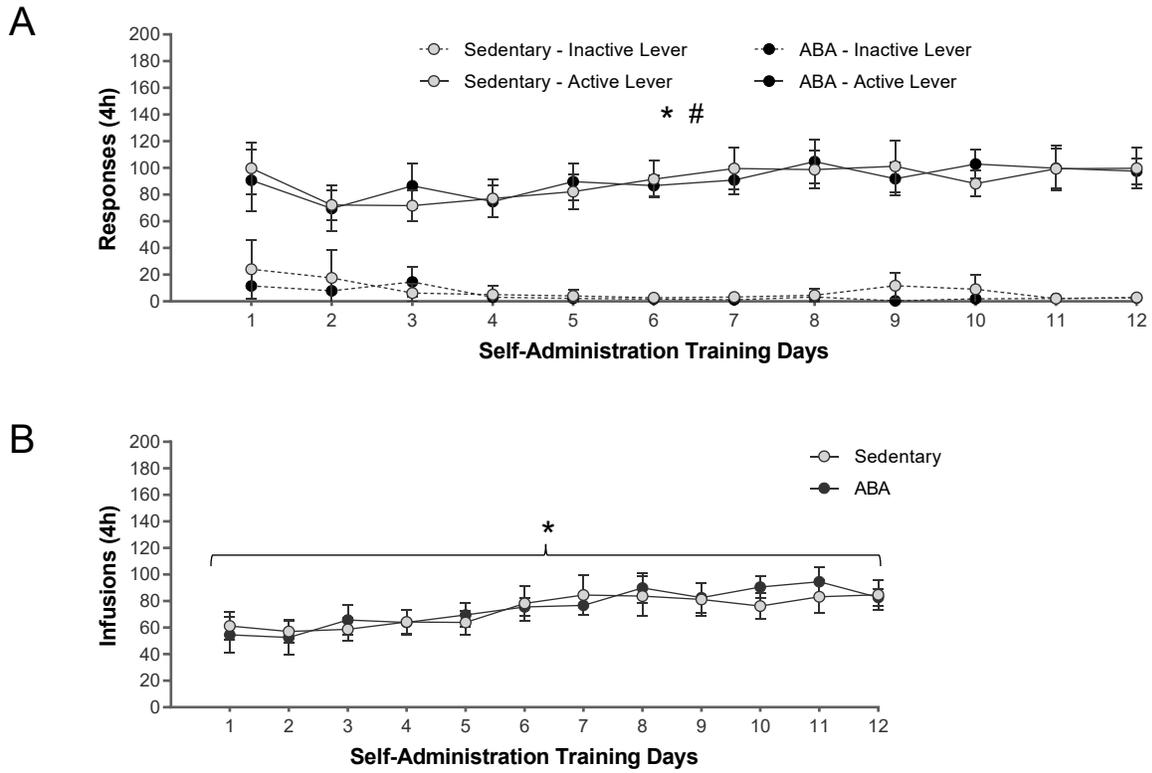


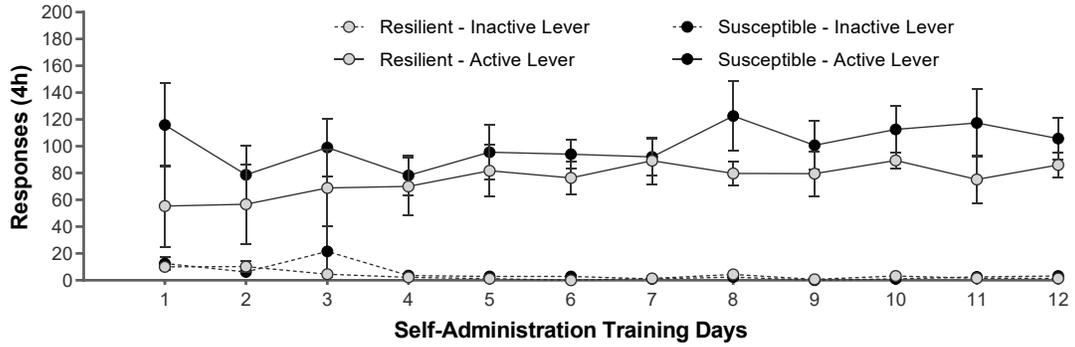
Figure 6.4. Responses and infusions during self-administration days in sedentary ($n = 12$) and ABA ($n = 12$) rats. (A) Mean (\pm SEM) of active and inactive lever presses across training days. $*p = .028$, main effect of *days* on active lever presses; $\#p < .001$, main effect of *days* on inactive lever presses. (B) Mean (\pm SEM) of infusions across training days. $*p < .001$, main effect of *days*.

Table 6.1.

Two-way ANOVA results for infusions, active lever presses and inactive lever presses across self-administration training days in sedentary and ABA rats.

Source	<i>df</i>	<i>F</i>	<i>p</i>	η_p^2
Infusions				
Days	4.25, 93.46	11.55	0.000	0.34
Activity Condition	1, 22	0.02	0.897	< 0.01
Days x Activity condition	4.25, 93.46	1.00	0.417	0.04
Active Lever Presses				
Days	4.57, 100.58	2.72	0.028	0.11
Activity Condition	1, 22	< 0.01	0.984	< 0.01
Days x Activity condition	4.57, 100.58	0.49	0.77	0.02
Inactive Lever Presses				
Days	3.96, 87.01	3.36	< .001	0.13
Activity Condition	1, 22	2.16	0.156	0.55
Days x Activity condition	3.96, 87.01	1.14	0.344	0.05

A



B

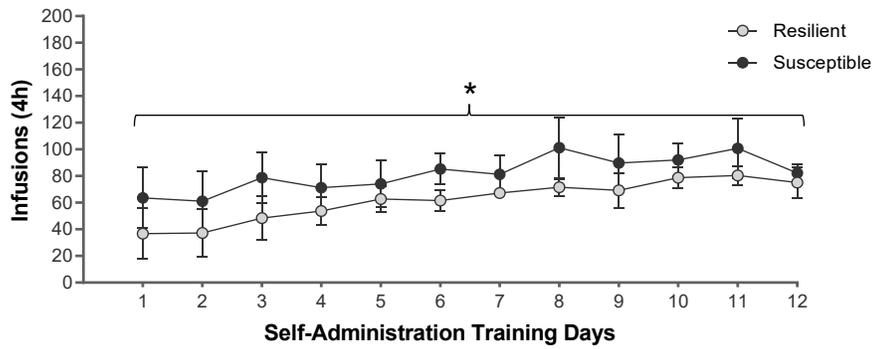


Figure 6.5. Responses and infusions during self-administration days in resilient ($n = 5$) and susceptible ($n = 7$) rats. (A) Mean (\pm SEM) of active and inactive lever presses across training days. (B) Mean (\pm SEM) of infusions across training days. $*p < .001$, main effect of days.

Table 6.2.

Two-way ANOVA results for infusions, active lever presses and inactive lever presses across self-administration training days in resilient and susceptible rats.

Source	<i>df</i>	<i>F</i>	<i>p</i>	η_p^2
Infusions				
Days	11, 110	7.37	< .001	0.42
Trait	1, 10	1.62	0.232	0.14
Days x Trait	11, 110	0.42	0.946	0.04
Active Lever Presses				
Days	11, 110	1.14	0.341	0.10
Trait	1, 10	1.18	0.304	0.11
Days x Trait	11, 110	0.74	0.700	0.07
Inactive Lever Presses				
Days	11, 110	1.42	0.172	0.13
Trait	1, 10	0.44	0.521	0.04
Days x Trait	11, 110	0.56	0.859	0.05

(Figure 6.6A). There was no difference, however, in Q_0 between the ABA and sedentary rats, $t(17) = -1.51, p = .149, d = 0.70$ (Figure 6.6B).

Resilient vs. susceptible rats. In ABA rats, there was a trend for higher averaged P_{Max} in resilient rats compared to susceptible rats, $t(9) = 2.24, p = .052, d = 1.41$ (Figure 6.6C). There was no difference in Q_0 between resilient and susceptible rats, $t(8) = 1.59, p = .150, d = 1.10$ (Figure 6.6D).

Extinction of cocaine seeking.

Sedentary vs. ABA rats. All rats showed extinction of cocaine-seeking behaviours throughout the extinction sessions (Figure 6.7A). When extinction sessions were collapsed, there was no statistically significant difference in active lever presses between sedentary and ABA rats. Interestingly, there was a statistically significant *days x activity condition* interaction demonstrating that ABA rats were more resistant to extinction compared to sedentary rats. On the first day of extinction, the mean (\pm SEM) of active and inactive lever presses for sedentary rats were 94.09 (11.67) and 38.91 (10.65) and 205.91 (40.85) and 41.27 (8.34) for ABA rats. On the final day of extinction, the mean (\pm SEM) of active and inactive lever presses for sedentary rats were 9.46 (2.52) and 3.55 (1.35) and 12.64 (3.54) and 6.00 (3.23) for ABA rats. Inactive lever presses decreased for all rats across extinction sessions. There were no statistically significant differences between sedentary and ABA rats in inactive lever presses, and no *days x activity condition* interaction. See Table 6.3 for detailed statistics.

Resilient vs. susceptible rats. When examining rats in the ABA condition only (Figure 6.7B), all rats showed extinction of cocaine-seeking behaviours across the extinction sessions. There were no differences between resilient and susceptible rats in overall number of active and inactive lever presses during extinction. There was a trending *days x activity condition* interaction suggesting that resilient rats may have been more resistant to extinction compared to susceptible rats. On the first day of extinction, the mean (\pm SEM) of active and inactive lever presses for resilient rats were 303.00 (99.95) and 54.25 (21.34) and 150.43 (10.24) and 33.86 (5.20) for susceptible rats. Under the final day of extinction, the mean (\pm SEM) of active and inactive lever presses for resilient rats were 54.25 (21.34) and 2.50 (2.18) and 15.43 (4.80) and 8.00 (4.91) for susceptible rats. See Table 6.4 for detailed statistics.

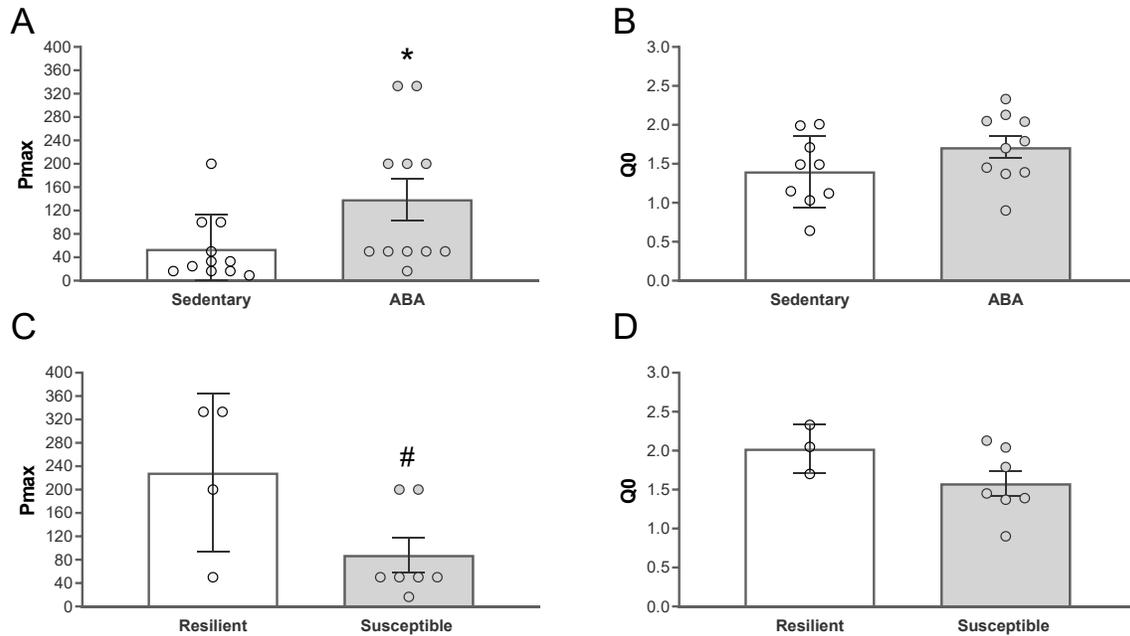


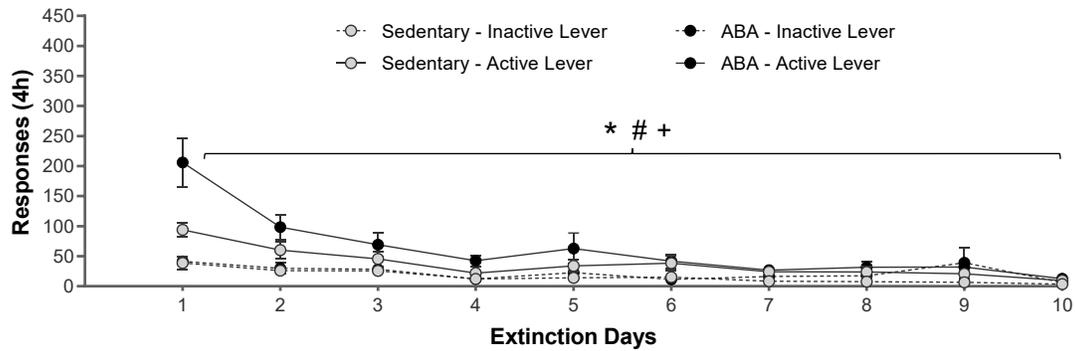
Figure 6.6. P_{max} and Q_0 averaged across 4 self-administration test sessions in sedentary ($n = 11$) versus ABA ($n = 11$) rats and in ABA-resilient ($n = 4$) versus ABA-susceptible rats ($n = 7$). (A) Mean (\pm SEM) of averaged P_{Max} in sedentary and ABA rats. $*p = .046$, $d = .91$, statistically significant between-group difference. (B) Mean (\pm SEM) of averaged Q_0 in sedentary and ABA rats. (C) Mean (\pm SEM) of averaged P_{Max} in resilient and susceptible rats. $\#p = .052$, $d = 1.41$, trending between-group difference. (D) Mean (\pm SEM) of averaged Q_0 in resilient and susceptible rats. P_{max} = price at which maximum work is performed. Q_o = preferred consumption when price is negligible.

Table 6.3.

Two-way ANOVA results for infusions, active lever presses and inactive lever presses across extinction days in sedentary and ABA rats.

Source	<i>df</i>	<i>F</i>	<i>p</i>	η_p^2
Active Lever Presses				
Days	2.62, 52.32	24.58	< .001	0.55
Activity Condition	1, 20	2.83	0.108	0.12
Days x Activity condition	2.62, 52.32	4.10	0.014	0.17
Inactive Lever Presses				
Days	2.45, 49.02	4.80	< .001	0.19
Activity Condition	1, 20	0.76	0.395	0.04
Days x Activity condition	2.45, 49.02	1.11	0.348	0.05

A



B

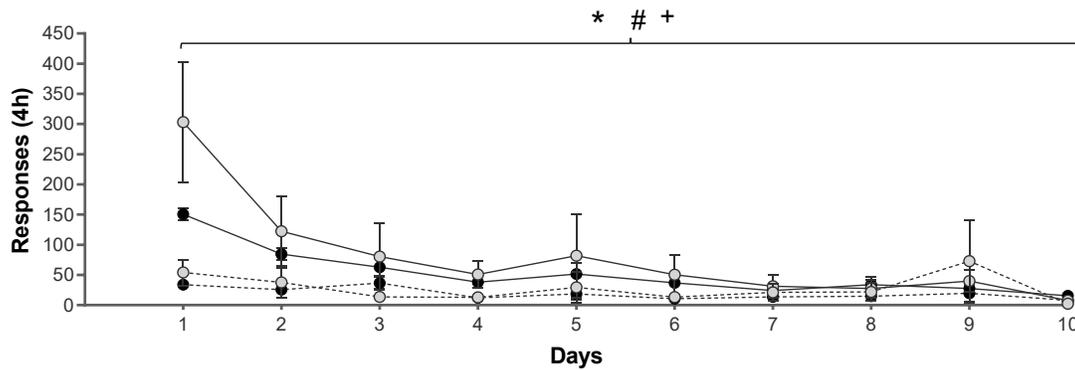


Figure 6.7. Active and inactive lever presses across extinction days (A) in sedentary ($n = 11$) and ABA ($n = 11$) rats. $*p < .001$, main effect of *days* on active lever presses; $\#p = .014$, *days x activity condition* interaction on active lever presses; $+ p < .001$, main effect of *days* on inactive lever presses, (B) in resilient ($n = 4$) and susceptible ($n = 7$) rats. $*p < .001$, main effect of *days* on active lever presses; $\#p = .079$, trending *days x activity condition* interaction on active lever presses; $+ p = .009$, main effect of *days* on inactive lever presses.

Table 6.4.

Two-way ANOVA results for infusions, active lever presses and inactive lever presses across extinction days in resilient and susceptible rats.

Source	<i>df</i>	<i>F</i>	<i>p</i>	η_p^2
Active Lever Presses				
Days	2.59, 23.26	19.19	< .001	0.68
Trait	1, 11	0.27	0.270	0.62
Days x Trait	2.59, 23.26	2.66	0.079	0.29
Inactive Lever Presses				
Days	1.57, 14.17	2.66	0.009	0.23
Trait	1, 9	0.33	0.583	0.04
Days x Trait	1.57, 14.17	1.37	0.217	0.13

Effects of ABA on cue-induced reinstatement of cocaine-seeking.

Sedentary vs. ABA rats. Cue-induced reinstatement for both sedentary and ABA rats can be seen in Figure 6.8A-B. All rats showed cue-induced reinstatement of cocaine seeking as can be seen by the increase in active lever presses from the last three sessions of extinction to the reinstatement session. There were no statistically significant differences between sedentary and ABA rats in cue-induced reinstatement and no *session x activity condition* interaction. There were no differences in inactive lever presses between the extinction and reinstatement sessions or between sedentary and ABA rats. There were also no significant interactions for inactive lever presses. See Table 6.5 for detailed statistics.

Resilient vs. susceptible rats. Cue-induced reinstatement for resilient and susceptible rats can be seen in Figure 6.8C-D. A closer examination of rats in the ABA condition revealed that all rats showed higher active lever presses, but not inactive lever presses, during the reinstatement session compared to during extinction. There were, however, no statistically significant differences between resilient and susceptible rats in either active or inactive lever presses and no statistically significant interactions. See Table 6.6 for detailed statistics.

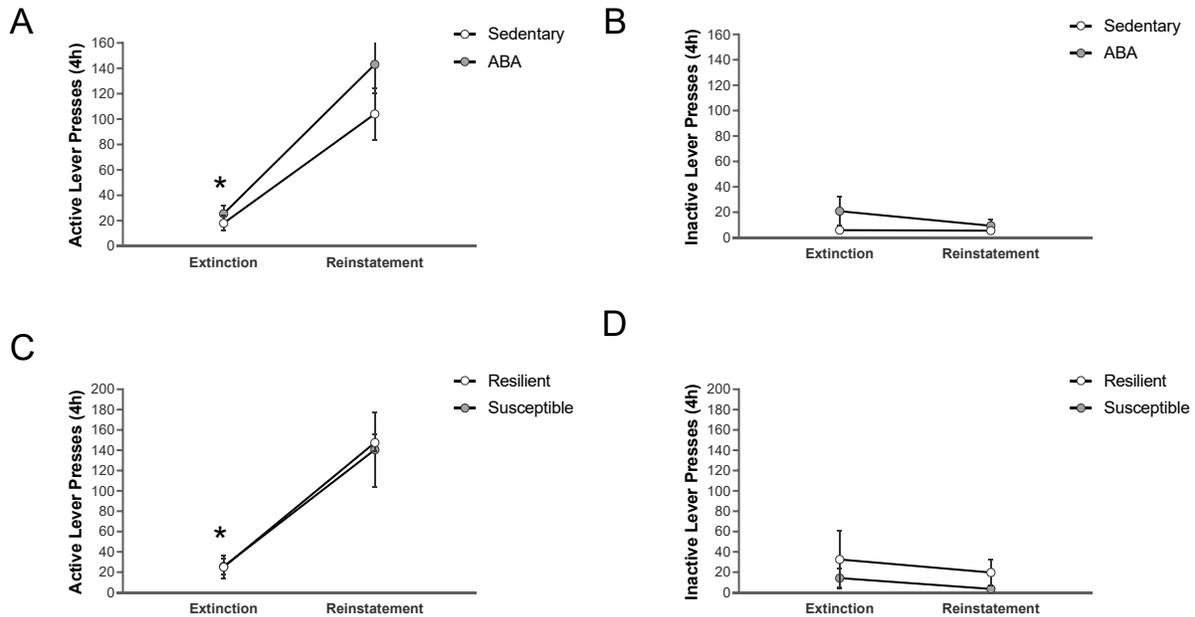


Figure 6.8. Active and inactive lever presses during extinction (averaged across last 3 sessions of extinction) versus cue-induced reinstatement in sedentary ($n = 11$) versus ABA ($n = 11$) rats and in ABA-resilient ($n = 4$) versus ABA-susceptible rats ($n = 7$). (A) Mean (\pm SEM) of active lever presses in sedentary and ABA rats. $*p < .001$, main effect of *session*. (B) Mean (\pm SEM) of inactive lever presses in sedentary and ABA rats. (C) Mean (\pm SEM) of active lever presses in resilient and susceptible rats. $*p = .002$, main effect of *session*. (D) Mean (\pm SEM) of inactive lever presses in resilient and susceptible rats.

Table 6.5.

Two-way ANOVA results for active and inactive lever presses from extinction to reinstatement in sedentary and ABA rats.

Source	<i>df</i>	<i>F</i>	<i>p</i>	η_p^2
Active Lever Presses				
Session	1, 20	44.02	< .001	0.69
Activity Condition	1, 20	1.96	0.177	0.09
Session x Activity condition	1, 20	1.06	0.316	0.05
Inactive Lever Presses				
Session	1, 20	2.33	0.143	0.10
Activity Condition	1, 20	1.39	0.252	0.07
Session x Activity condition	1, 20	2.01	0.172	0.09

Table 6.6.

Two-way ANOVA results for active and inactive lever presses from extinction to reinstatement in resilient and susceptible rats.

Source	<i>df</i>	<i>F</i>	<i>p</i>	η_p^2
Active Lever Presses				
Session	1, 9	19.82	0.002	0.69
Trait	1, 9	0.02	0.904	< .01
Session x Trait	1, 9	0.02	0.890	0.02
Inactive Lever Presses				
Session	1, 9	1.98	0.193	0.18
Trait	1, 9	1.13	0.315	0.11
Session x Trait	1, 9	0.02	0.901	< .01

Discussion

Individuals with AN show high prevalence of substance use disorders including cocaine use and abuse. Furthermore, studies examining reward processing and neurobiological reward mechanisms have provided evidence for reward deficits in AN. Altered reward functioning has also been reported in rodents exposed to ABA, but no studies have examined drug taking behaviours in this model. Therefore, the present study was the first to use animal models of addiction and relapse in rats previously exposed to the ABA model. It was hypothesized that rats with a history of ABA compared to sedentary rats, would be more motivated to work for cocaine, more resistant to extinction, and would show higher cue-induced reinstatement. Importantly, we expected that these results would be amplified in ABA-susceptible rats compared to ABA-resilient rats.

Comparison of the two restriction phases

In the present study, rats in the ABA condition were exposed to two phases of ABA, separated by a week of recovery. This protocol was used to mimic the relapsing nature of AN. Furthermore, this approach was also thought to enhance individual differences in ABA response as this has been shown to be the case in C57/BL6 mice exposed to two bouts of ABA versus one (Chowdhury et al., 2013). The exacerbation of individual differences in ABA response was important here as we aimed to examine differences between resilient and susceptible rats. Contrary to what was hypothesized, however, we found that individual differences in ABA response was reduced during the second ABA phase compared to the first. More specifically, all rats reduced their running wheel activity, increased their food intake, and lost less weight during the second restriction phase compared to the first. Furthermore, susceptible rats particularly increased their food intake during the second restriction phase indicating that they were adapting to the ABA procedure which rendered them less susceptible. These findings are partially inconsistent with findings in C57/BL6 mice in which two restriction phases increases individual differences (Chowdhury et al., 2013). Our results, however, are consistent with our previous attempts at using two restriction phases. Our overall experiences using a two-bout model in female Sprague Dawley rats will be further discussed in the general discussion.

Prior experience of ABA increased motivation to work for cocaine but did not increase cocaine-self administration

It was hypothesized that previous experience of ABA would result in higher cocaine self-administration and a greater willingness to work for the drug compared to sedentary rats with no history of ABA. Analyses of infusions and lever presses during self-administration training revealed no between-group differences suggesting that a history of ABA did not appear to increase cocaine self-administration. Consistent with our hypothesis, however, the unit-price at which maximum responding occurred (P_{Max}) was higher in ABA rats compared to sedentary rats during self-administration testing. In other words, ABA rats were willing to work harder to acquire cocaine (Oleson & Roberts, 2009).

Increased motivation to work for cocaine in rats with a previous history of ABA is in line with findings from the human literature whereby lifetime prevalence of substance use disorders is heightened in individuals with AN. Given the limited research on addiction in AN, little is known about the prevalence of addiction across the course of AN and whether the risk persists following recovery from the disorder. Though an extrapolation, our results suggest that addiction risk may persist after recovery as the heightened motivation to work for cocaine was observed in rats that were no longer under ABA conditions.

As the present experiment was the first to examine drug self-administration in the ABA model, no direct comparisons can be drawn. Our findings can, however, be considered in light of relevant studies of cocaine self-administration. For instance, Specker, Lac, and Carroll (1994) investigated cocaine self-administration in rats that had been previously exposed to a procedure designed to model binge eating. In this model, rats were exposed to three periods of food deprivation, followed by weight recovery. Rats were then injected with butorphanol tartrate, an opioid that stimulates binge-feeding. After 1–2 months of full recovery, rats were trained to intravenously self-administer cocaine. The authors observed a trend (did not reach statistical significance) whereby the rate of acquisition of cocaine was quicker in rats with a history of both food deprivation and binge-feeding, compared to rats that binged but did not have a history of food deprivation. This would suggest that the experience of food deprivation modulates the effects of binge-feeding on later cocaine self-administration. Our results add to this finding by suggesting that the experience of food restriction alone is also not sufficient to result in later changes in cocaine self-administration as sedentary rats that did experience food restriction were less motivated to work for cocaine compared to the ABA rats that experienced the combination

of food restriction and wheel running. As we know from our previous studies, rats exposed to ABA display hyperphagia during recovery which can be considered binge-eating. Thus, our results are in line with those of Specker, Lac, and Carroll (1994) suggesting that a history of severe food restriction and binge-feeding leads to faster acquisition of cocaine self-administration or, in our case, higher motivation to work for cocaine. This is interesting in light of Herzog et al. (2006)'s finding in humans that the prevalence of substance use disorders was higher in individuals who binged (i.e., BN and AN-BP) compared to individuals who did not binge (i.e., AN-R).

Another study to consider in the interpretation of our results of higher P_{Max} in ABA rats compared to sedentary rats is the study by Oleson, Richardson, and Roberts (2011) on the behavioural economic assessment of price and cocaine consumption. In this study, D-AMPH injections prior to cocaine self-administration increased P_{Max} in rats. D-AMPH is a stimulant drug that increases extracellular levels of DA in the NAcc, an important region of the mesolimbic DA system which is involved in reward. Their results therefore suggest that manipulation of mesolimbic DA circuitry led to later enhanced motivation to obtain cocaine. Thus, it is possible that alterations in DA functioning in ABA may have had prolonged effects on willingness to obtain cocaine in the present study. In other words, previous experience with anorexia-like symptoms may simulate reward-related changes in the dopaminergic system, leading to stronger appetitive responses to cocaine self-administration.

A history of ABA resulted in resistance to extinction

We hypothesized that ABA rats would show greater resistance to extinction following a 15-day withdrawal period, compared to sedentary rats. We observed higher active lever presses in ABA rats at the start of extinction sessions, compared to sedentary rats. Towards the end of extinction, however, active lever presses were diminished in both groups. These findings support the initial prediction that cocaine seeking would decrease more slowly in ABA rats. This corroborates Comer, Lac, Wyvell, Curtis, and Carroll (1995)'s findings on the effects of food deprivation in extinction of cocaine seeking. In this study, rats were divided into groups based on food deprivation level and time of feeding (pre- or post-testing). After 3 hours of extinction, rats with the greatest food deprivation showed increased cocaine-seeking when feeding occurred post-testing, suggesting that food restriction interferes with extinction learning. We propose that

previous experience with food restriction and running wheel activity may have lasting effects resulting in increased resistance to extinction.

One possible explanation for the observed resistance to extinction in ABA rats is that ABA induces long-lasting impairments in learning which interfere with extinction learning. Frintrop et al. (2018) exposed adolescent female rats to the ABA model and examined volume changes in the brain. ABA rats had a greater reduction in total brain volume in the cerebral cortex (6%) and corpus callosum (9%), compared to satiated control rats. Furthermore, the number of astrocytes in these areas were significantly reduced in ABA rats, compared with control rats. The researchers propose that astrocyte reduction may relate to neuropsychological deficits reported in AN, such as inflexibility and impaired learning. Allen, Jimerson, Kanarek, and Kocsis (2017) tested whether ABA was associated with impaired cognitive flexibility. Using an attentional set-shifting test, they found that ABA rats, unlike weight-loss paired control rats, showed a decrement in reversal learning. It is therefore possible that resistance to extinction observed here stems from impaired learning resulting from a history of ABA.

Prior experience with ABA had no effect on later cue-induced reinstatement of cocaine seeking

In a test of cue-induced reinstatement, we hypothesized that ABA rats would show a greater return to cocaine seeking, compared to sedentary rats. Active lever presses increased dramatically in both ABA and sedentary rats on reinstatement day, compared to the last days of extinction. Additionally, there were no differences in either activity groups' inactive lever presses on reinstatement day, compared with the last day of extinction indicating that the behaviour was specifically directed at cocaine seeking. Contrary to our hypothesis, there were no between-group differences in active lever presses. As such, a history of ABA did not appear to increase cocaine seeking in rats following re-exposure to previously drug-associated cues. Unfortunately, because this is the first study examining self-administration and relapse in the context of ABA, the magnitude of the difference between ABA and sedentary rats on reinstatement day ($d = 0.54$) is not comparable to any other studies. Therefore, Cohen's guidelines may be used as a reference point for effect sizes when there is no other comparison. The guidelines suggest that this value exceeds the minimum cut-off for a moderate effect

(Ferguson, 2009). This effect may not have been detected through significance testing as a result of low statistical power and high variability within groups and replication studies will be needed.

ABA-resilient rats trended towards higher motivation to work for cocaine and slower extinction to cocaine seeking compared to ABA-susceptible rats

The main goal of this experiment was to assess for differences between ABA-resilient and ABA-susceptible rats in cocaine-taking behaviours. In line with our hypotheses about sedentary and ABA rats, we expected that susceptible rats would be more motivated to work for cocaine, more resistant to extinction, and would show higher cue-induced reinstatement. Surprisingly, our data revealed a trend in the opposite direction whereby resilient rats seemed to have a higher P_{Max} and slower extinction compared to susceptible rats. One cannot ignore that these trends, if accurate, would suggest that the effects observed in ABA rats may, counterintuitively, be driven by the resilient rats rather than the susceptible rats. In other words, rats that had the most severe experience of ABA seemed less motivated to work for cocaine and extinguished cocaine seeking more rapidly. Unfortunately, interpretation of these results is limited by the very small sample sizes used (4 resilient rats, 7 susceptible rats). In addition to these results reaching trend-level only, which in and of itself warrants caution, the limited sample sizes render it difficult to draw any illegible conclusions about these data. The present study will need to be replicated with more animals to further dissect differences between resilient and susceptible rats.

Experience of ABA may interfere with the protective nature of wheel running on later cocaine-taking behaviours

In the present study, rats in the ABA condition had continuous access to the running wheel throughout the entire experiment (including during the cocaine phase) while rats in the sedentary condition remained sedentary throughout. There is a large amount of evidence suggesting that wheel running in rodents can serve as a protective factor against drug-taking behaviours whereby it may provide a hedonic substitution as an alternate reinforcer replacing drugs of abuse such as cocaine (Novak et al., 2012). For instance, wheel running can decrease oral consumption of AMPH in rats (Kanarek, Marks-Kaufman, D'Anci, & Przypek, 1995). Wheel running can also substantially reduce intravenous self-administration of cocaine in rodents (Cosgrove, Hunter, & Carroll, 2002; Smith, Schmidt, Iordanou, & Mustroph, 2008).

More specifically, Smith et al. (2008) propose that long-term wheel running can reduce the positive-reinforcing effects of cocaine in rats. Using a progressive ratio of schedule reinforcement, the researchers examined the effects of chronic running wheel activity on intravenous cocaine self-administration in rats. Breakpoints at both high (1.0 mg/kg/infusion) and low (0.3 mg/kg/infusion) doses of cocaine were lower in physically active rats, compared to sedentary rats. Based on these findings, one might expect that the ABA rats would have been protected against cocaine use by their running wheel activity, but we did not find this to be the case. Instead, ABA rats, regardless of their continued access to the running wheel compared to sedentary rats, showed more motivation to work for cocaine and slower extinction of cocaine seeking. This finding by no means negates the protective nature of physical activity on addiction-like behaviours but instead suggests that the protective effects of wheel running were unable to make up for the long-term effects of ABA on later cocaine self-administration and extinction. Although speculative, it is possible that the long-term effect of ABA on later cocaine-taking behaviours would have been even more robust in a design where all rats had been sedentary during the cocaine phase.

Methodological considerations for future studies

The present experiment was the first to examine drug self-administration in rats exposed to ABA and provides a protocol that can now be used to further the understanding of the neurobiological and behavioural mechanisms implicated in the development and course of co-occurring AN and substance use disorders. It did, however, have some limitations that should be carefully considered in future designs. This specific experiment was limited by the small sample size. Due to ethical and logistical considerations that limit the number of rats that can be used in experiments, such small samples are common in animal research. Importantly, small sample size minimizes statistical power and it is possible that some of the effects of ABA on cocaine self-administration and cocaine-seeking may not have been detected through significance testing as a consequence. Furthermore, the present experiment included one control condition only: sedentary rats. Additional control conditions such as a sated-active group and a sated-sedentary group would have made it possible to draw conclusions on the effect of food restriction and/or wheel running alone on later cocaine use. Given the reportedly protective nature of wheel running on cocaine self-administration, it would be interesting to either remove access to the

running wheel for all rats during the cocaine phase of the experiment or to include an additional ABA group that was sedentary during the cocaine phase. Another design-related limitation was that restriction phases were capped at 7 days for the first restriction phase and 9 days during the second restriction phase. These cut-offs were chosen as the most susceptible rats had begun to reach the starvation criterion (25% weight loss) at those time points and needed to begin recovery. Due to complicated logistics, we were unable to use a staggered procedure whereby rats would have started the cocaine phase depending on their speed of weight loss. This resulted in a somewhat blunted ABA effect as some rats would have needed more days under ABA to reach starvation criterion. As such, the ABA effect was not as severe as it could have been using a staggered procedure which may have reduced effects on later cocaine use.

CHAPTER 7. GENERAL DISCUSSION

The general goal of this dissertation was to further the understanding of the ABA model of AN-like behaviours and, more specifically, investigate for behavioural and neurobiological differences between rats that are most susceptible to the development of ABA and those that are more resilient. In Chapter 3, we attempted to establish the use of the ABA model for the first time in our laboratory. We found that ABA reliably develops in both male and female adult rats and that female rats were particularly susceptible to ABA showing higher overall running activity, a larger increase in running activity in response to food restriction, and more rapid weight loss. The ABA effect using a 60 min/day feeding schedule was in fact so robust in females that starvation criterion was reached within 5 days, making it difficult to run any lengthy behavioural experiments during this time. Our first efforts to use behavioural tests (e.g., FST, set-shifting test) during the ABA paradigm revealed that it is possible to do so without interfering with ABA development. We also observed significant individual variability in rats' susceptibility to ABA which merited further investigation.

In Chapter 4, we aimed to further characterize ABA resilience and susceptibility by assessing baseline anxiety- and depression-like behaviours, AMPH-induced locomotor activity as an index of mesolimbic DA activity, and response to pharmacological treatment. Using a median split of percent of initial body weight after 4 days of ABA to identify resilient and susceptible rats, we repeatedly found baseline running wheel activity to be a reliable predictor of ABA susceptibility. Susceptible rats were also found to show longer latency to immobility and less immobility time in the FST before ABA (traditionally interpreted as less depression-like behaviour). Using AMPH-induced locomotor activity, we found no evidence of trait differences before or after ABA in mesolimbic DA. Finally, we found that treatment with OLZ, an atypical antipsychotic drug, was effective in reducing running wheel activity in both resilient and susceptible rats undergoing ABA.

In Chapter 5, we compared general neural activity between resilient and susceptible rats in several key brain areas using c-Fos immunohistochemistry and found that ABA-susceptible rats, compared to ABA-resilient rats, appeared to show more neural activity in the prelimbic and infralimbic cortices of the PFC and lower activity in the NAcc shell. We followed up on these findings by assessing response inhibition, a facet of impulsivity that is dependent on PFC

functioning but failed to provide evidence for differences in impulsivity between resilient and susceptible rats.

Finally, in Chapter 6, we sought to examine the effect of a history of ABA on later cocaine self-administration. We were particularly interested in determining whether rats that are most susceptible to ABA development would also be more susceptible to addiction-like behaviours. Our main finding was that rats with a history of ABA showed more motivation to work for cocaine and were more resistant to extinction compared to sedentary rats that had not experienced ABA. With regards to trait differences, our results were contrary to our hypotheses whereby susceptible rats seemed to be less motivated to work for cocaine and showed less resistance to extinction of cocaine seeking. Interpretation of these latter findings were limited by small sample sizes.

ABA susceptibility: Putting the pieces together

Identifying and predicting ABA susceptibility. Typically, studies using the ABA model have used classical designs with a treatment and control condition whereby rats exposed to the ABA procedure (food restriction and wheel access) are compared to control rats that are active, but sated and/or to sedentary, but food restricted rats. Using such designs in our early experiments, the individual variability in severity of weight loss within the ABA condition became apparent. We thus turned our focus towards ABA resilience and susceptibility with the rationale that identifying those animals that are most susceptible to ABA may shed light onto individual factors involved in AN susceptibility in humans. After all, only 0.9% of the general population experience AN in their lifetime (Keski-Rahkonen et al., 2007) and certainly not every individual engaging in physical activity and caloric restriction develops AN.

Median split of percentage of initial body weight. In all experiments in this dissertation that examined resilience and susceptibility, we used a median split of percentage of initial body weight after a few days of ABA (typically after 4 days) to retrospectively identify resilient and susceptible rats. The first rats to reach 75% of their initial body weight were considered susceptible while the remaining rats, many of which managed to maintain their body weight above the starvation criterion, were considered resilient. The identification of this resilient and susceptibility trait in adult Sprague Dawley female rats has recently been replicated by Milton et al. (2022). Our finding is also consistent with reports in adult female mice whereby a subset of

mice are unable to stabilize their weight under ABA conditions compared to resilient mice that manage to do so (Beeler et al., 2021). Our resilience-susceptibility differentiation could certainly be further strengthened by using subjects at both extremes and omitting the rats that fall closer to the median, though such an approach requires a large number of animals. Regardless, we found the median split based on percentage of initial body weight to be a reliable way of identifying susceptibility as rats that were labeled as susceptible were also the rats that showed the largest food restriction-induced hyperactivity (increase in running wheel activity from habituation to restriction) and the highest levels of wheel running both during ABA and at baseline.

Food intake. Repeatedly we did not find differences between resilient and susceptible rats in food intake during ABA. Instead, we consistently found that susceptible rats compared to resilient rats (and ABA rats compared to sedentary rats) either ate the same amount or more. Their food intake was, however, insufficient to compensate for their increased energy expenditure resulting in the rapid weight loss. This appears to differ from what has been observed in mice whereby resilient mice consume more food than susceptible mice when exposed to ABA (Beeler et al., 2021). Our results suggest that, in rats, the term “anorexia” in the ABA model refers to insufficient caloric intake to maintain a healthy body weight rather than caloric restriction relative to sedentary rats or ABA-resilient rats.

Baseline running wheel activity. Level of running wheel activity across all our experiments consistently and positively predicted ABA susceptibility. At baseline (i.e., prior to ABA), susceptible rats showed higher levels of running wheel activity compared to resilient rats. This finding suggests that baseline running wheel activity can be used as a predictor of ABA susceptibility and is consistent with reports from other researchers (Barbarich-Marsteller et al., 2013; Milton et al., 2018; Perez-Leighton et al., 2014; Pjetri et al., 2012). This is of particular interest for study designs in which susceptibility must be identified prior to the ABA procedure. For instance, in the present dissertation, we opted to expose rats to two bouts of ABA – a first one to identify susceptible rats, and a second to examine the effect of a manipulation (e.g., OLZ treatment, chemogenetic inhibition). Knowing now that Sprague Dawley female rats adjust their behaviour and show less variability in a second bout of ABA (discussed further in “methodological considerations”), using baseline running wheel activity to predict susceptibility would have been beneficial.

A note on the microstructure of running wheel activity. ABA susceptible rats not only showed higher baseline activity compared to resilient rats, but they also showed more activity during ABA. While the microstructure of running wheel activity was not a focus of the present dissertation, there is reason to believe that the increased activity in susceptible rats during ABA is driven by increases in PPA. Studies examining the microstructure of running wheel activity during ABA have long pointed towards FAA as an important feature of ABA (Wu et al., 2014). FAA refers to the wheel running peaking in the hours before scheduled feeding (typically 3-4 hours prior to scheduled feeding) and a decrease in FAA in ABA studies is often interpreted as improvement of the anorectic state though not always correlated with body weight increase or higher survival rate (Atchley & Eckel, 2006; Hillebrand, Van Elburg, et al., 2005; Klenotich et al., 2012; Mistlberger, 1994; Verhagen, Luijendijk, Korte-Bouws, et al., 2009). In the first study examining ABA-susceptibility in relation to the microstructure of wheel running during ABA, Wu et al. (2014) investigated for correlations between percentage of weight loss and FAA, PPA, nocturnal activity, and feeding activity. Based on previous reports of FAA being a central component of ABA, the authors hypothesized that susceptible rats would show higher FAA compared to resilient rats. Surprisingly, however, they did not find FAA to be proportional to susceptibility. Instead, they found PPA to be a better predictor of weight loss in ABA. While both FAA and PPA increased over time in all rats undergoing restricted feeding, only PPA was increasing at higher rates in susceptible rats compared to resilient rats. Wu et al. (2014) argue that FAA is a more common phenomenon in response to ABA whereas PPA is a more distinctive feature resulting in higher weight loss. Shifts in circadian activity have also been reported in adult female mice exposed to ABA whereby ABA induces a shift from dark activity to light activity which is more pronounced in susceptible mice compared to resilient mice (Beeler et al., 2021). The above findings are consistent with our own observations in a preliminary study (see Appendix 1) in which we attempted to examine the relation between ABA susceptibility and running wheel activity at different times of the day. While this experiment had important limitations, our preliminary findings also suggested that PPA, rather than FAA, may be a distinguishing feature of ABA susceptibility.

Depression- and anxiety-like behaviours in ABA susceptibility. Given the high cooccurrence of AN and mood-related disorders such as depression and anxiety disorders (de Gortari, Alvarez-Salas, García-Luna, Soberanes-Chávez, & Valdés-Moreno, M Pineda, 2016),

depression- and anxiety-like behaviours were assessed several times throughout this dissertation. Important parameters varied in each experiment (e.g., sex, strain, timing of test) and the results pertaining to each of these experiments are discussed in their respective chapters. The question of differences in anxiety- and depression-like behaviours between resilient and susceptible rats, however, was addressed in several experiments and merits further elaboration. Trait differences in anxiety- and depression-like behaviours were assessed using the EPM and FST, respectively, in experiment 2.1 (Chapter 4) and experiment 3.2 (Chapter 5). Our results suggest that resilient and susceptible rats may differ in depression-like behaviours in the FST, but not in anxiety-like behaviours in the EPM.

In experiment 2.1, it was hypothesized that susceptible rats, compared to resilient rats, would show more depression-like behaviours (e.g., shorter latency to immobility, more time spent immobile) at baseline (i.e., prior to ABA). Instead, we found that susceptibility was associated with longer latency to immobility and less time spent immobile. This result was not replicated in experiment 3.2 where we failed to observe a difference between resilient and susceptible rats during FST prior to ABA. It should be noted, however, that experiment 3.2 was our only experiment where the resilience-susceptibility trait was less robust (susceptible rats showed more hyperactivity than resilient rats, but they did not lose more weight) which may explain the absence of trait differences in FST. Our findings from experiment 2.1 should therefore be replicated in future studies. Based on the classical interpretation of behaviour in the FST, our results would suggest that ABA-susceptible rats, compared to resilient rats, showed less depression-like behaviours or, more specifically, less behavioural despair, at baseline before ABA. This contradicts the human literature in which depression has been shown to be highly comorbid with AN and occasionally present prior to the onset of illness (Godart et al., 2015).

While the FST is considered the gold standard for assessing depression-like behaviours in rodents, the classical interpretation that immobility reflects behavioural despair and that escape-directed behaviours represent the absence of a depression-like phenotype has been challenged (Anyan & Amir, 2018; Bogdanova et al., 2013). As anxiety and depression tend to co-occur in humans, one would expect that depression-like behaviours in the FST would correlate with anxiety-like behaviour on the EPM. Instead, FST results have been shown to be either uncorrelated with EPM (Ho, Eichendorff, & Schwarting, 2002) or negatively correlated (i.e.,

higher anxiety-like behaviours in EPM associated with lower depression-like behaviours in FST; Estanislau et al., 2011). Anyan and Amir (2018) suggest that escape-related behaviours in the FST may be driven by anxiety rather than the absence of a depression-like phenotype. From this lens, it is possible that the increased escape-related behaviours observed in susceptible rats is a means of coping with heightened anxiety

While the interpretation of higher escape-related behaviours in the susceptible rats as a reflection of higher baseline anxiety is a plausible one, it should be considered with caution because we did not find any differences in anxiety-like behaviours in the EPM between resilient and susceptible rats. Instead, our results suggest that resilient and susceptible rats do not differ on the EPM prior to the onset of ABA, during ABA, or following recovery from ABA. Our finding of absence of a trait difference in anxiety-like behaviours at baseline is consistent with reports from Wable et al. (2015) whereby hyperactivity in mice during ABA was not correlated with anxiety-like behaviours in the open field test at baseline. Contrary to our findings, however, these authors reported that hyperactivity was associated with more anxiety-like behaviours in the EPM during ABA (Wable et al., 2015). There have also been reports of elevated anxiety-like behaviours in the EPM in adulthood in rats that had previously undergone ABA during adolescence (Kinzig & Hargrave, 2010; Lee & Kinzig, 2017) which was not replicated in the present dissertation. It should be noted that the EPM is known to be particularly sensitive to variations in environmental and procedural variables including ambient light (Bertoglio & Carobrez, 2002), presence of other subjects or investigators (Sorge et al., 2014), starting position (Pellow, Chopin, File, & Briley, 1985), trial length (Carobrez & Bertoglio, 2005), time of day (Andrade, Tomé, Santiago, Lúcia-Santos, & De Andrade, 2003), and prior handling (Andrews & File, 1993). Studies that have examined the between-laboratory replication of results from EPM have found that, even when all variables appear to be controlled, there is notable variability in results (Crabbe, Wahlsten, & Dudek, 1999). The within-subject test-retest reliability of the EPM is poor and anxiolytic drugs have been shown to reduce the behavioural measures of anxiety only during the first exposure to the test (File, Mabbutt, & Hitchcott, 1990). Based on these findings, some authors have suggested that anxiety-like behaviours in the EPM most likely represents state, rather than trait anxiety (Andreatini & Bacellar, 2000). It is thus possible that the EPM did not have the sensitivity required to detect differences between resilient and susceptible rats at baseline. Hoffman (2016) even suggests that the EPM might be a test more relevant to the

endophenotype of behavioural inhibition rather than trait anxiety as it represents the conflict between the motivation to explore and the motivation to avoid potentially dangerous situations. In this light, our EPM results would be consistent with results from our impulsivity experiment in which we failed to observe any differences between resilient and susceptible rats in response inhibition. Given the limited data on anxiety in ABA and the discrepancy between our results and the limited literature, the question of anxiety as it relates to ABA susceptibility merits further investigation and should include additional measures of anxiety.

Another more recent interpretation of the FST is that immobility is an adaptive learned response reflecting a transition from active to passive coping strategies when a stressful condition is appraised as inescapable or uncontrollable (De Kloet & Molendijk, 2016). From this perspective, the forced swim stressor that takes place on the first day of testing teaches animals that escape is impossible resulting in consolidation of acquired immobility which is considered an evolutionary-conserved energy-sparing survival mechanism. In the present dissertation, rats that are susceptible to ABA are those that fail to preserve their energy expenditure in the context of food restriction, resulting in accelerated weight loss. These same rats showed less immobility time and longer latency to immobility in the FST compared to ABA-resilient rats. One possible interpretation is therefore that the reduced immobility time in the FST for susceptible rats reflects a disruption in energy-sparing survival mechanisms which yields these rats more susceptible to ABA.

A third potential explanation for the finding that susceptible rats, compared to resilient rats, display less immobility time and a longer latency to immobility at baseline is that these differences simply reflect hyperactivity. Indeed, spontaneous physical activity has been reported to predict running wheel activity and ABA susceptibility (Perez-Leighton et al., 2014; Pjetri et al., 2012). It is thus possible that the behaviour of susceptible rats in the FST is another display of higher spontaneous activity in these rats. This view is supported by a study showing that rats that display the most running wheel activity during the light phase have a longer latency to immobility and spend less time immobile in the FST (Anyan, Verwey, & Amir, 2017). In the present dissertation, spontaneous physical activity was not explicitly assessed. Our indirect measures of spontaneous physical activity, however, did not reveal any differences between resilient and susceptible rats. More specifically, we did not find any differences between resilient

and susceptible rats in total closed arm entries –a measure of general locomotor activity – in the EPM (discussed in Chapter 4, experiment 2.1). Furthermore, we did not observe trait differences in activity in locomotor activity monitoring chambers in experiment 2.2 (Chapter 4). These results, further discussed in Chapter 4, do not suggest that ABA-susceptible rats necessarily show more spontaneous activity in the running wheel making it unlikely that the increased activity in the FST results from more locomotor activity in these rats.

In summary, we did not find evidence of increased anxiety-like behaviours (using the EPM) in susceptible rats at baseline, during, or after ABA. We did, however, find that susceptible rats differed from resilient rats in the FST whereby they had a longer latency to immobility and spent less time immobile. While the classical interpretation of these findings would be that resilient rats displayed more depression-like behaviours (i.e., behavioural despair) than susceptible rats, we offer three alternative interpretations. First, we suggest that the increased escape-related behaviours in the FST in susceptible rats may be driven by increased anxiety rather than the absence of a depression-like phenotype. A second interpretation is that reduced immobility in the FST in susceptible rats may reflect a disruption in energy-sparing survival mechanisms. Finally, a third interpretation worth considering is that lower immobility time in the FST in susceptible rats may be a display of higher spontaneous activity in these rats compared to resilient rats.

The role of DA in ABA susceptibility. DA has long been believed to play an important role in AN. Following the initial proposition that increased DA plays a central role in AN symptomatology (Barry & Klawans, 1976) many researchers investigated DA functioning in acute AN and recovered individuals (e.g., Broft et al., 2015; Frank et al., 2005; Kaye et al., 1999; Kontis & Theochari, 2012). Despite decades of research, the direction of change in DA in AN remains obscure and the scarcity of prospective studies makes it difficult to determine whether these changes precede illness onset. In an effort to contribute to unraveling this puzzle, we sought to examine whether DA may play a role in ABA susceptibility.

Our two experiments aimed at investigating the role of DA in ABA susceptibility. In experiment 2.2 (Chapter 4), locomotor activity following varying doses of AMPH was used as an index of mesolimbic DA activity. These tests took place following ABA exposure and, in a different set of rats, prior to ABA to assess for potential baseline differences between resilient

and susceptible rats. No differences were observed between resilient and susceptible rats in response to the AMPH challenges, suggesting that there are no trait differences in mesolimbic DA activity at baseline or following exposure to ABA. In experiment 2.3 (Chapter 4), rats were administered chronic OLZ, an atypical antipsychotic drug acting as an antagonist on several DA receptors, during ABA to examine whether OLZ would prolong survival, particularly in susceptible rats. Rather than using baseline activity to assign rats to treatment groups, rats underwent two phases of ABA – a first to identify susceptibility and a second to test OLZ treatment. Unfortunately, we found that all rats, regardless of treatment, showed an attenuated ABA response during the second bout. This interfered with our ability to draw conclusions about the effect of OLZ treatment on ABA susceptibility. We did find, however, that OLZ reduced running wheel activity in both resilient and susceptible rats.

Recent publications on DA in ABA. Since the completion of the experiments presented in this dissertation, two studies aimed at investigating the role of DA in ABA have been published. Beeler et al. (2021) first examined and compared ABA development in adolescent and young adult C57BL/6N female mice. Consistent with our findings across the present dissertation, the authors found important individual differences in ABA susceptibility in adult mice whereby some mice managed to maintain their body weight while others increased their running wheel activity and failed to increase their food intake resulting in accelerated weight loss. The authors also found that this trait difference was only present in adult mice as all adolescent mice exposed to ABA were susceptible (i.e., no adolescent mice were able to maintain their body weight). The heightened susceptibility in adolescent versus adult mice did not appear to be driven by baseline differences in striatal DA. Importantly in the context of our discussion on DA, Beeler et al. (2021) also sought to investigate DA's contribution to ABA susceptibility by comparing ABA development in DA transporter (DAT) knockdown mice (i.e., resulting in hyperdopaminergia) compared to wild-type control mice. There were no differences in running wheel activity across baseline days between DAT knockdown mice and wild-type mice. During ABA, however, DAT knockdown mice showed accelerated weight loss and shorter survival time compared to wild-type mice. Furthermore, all DAT knockdown mice displayed ABA susceptibility while some wild-type mice exhibited resilience. In line with Barry and Klawans (1976) initial proposition of a role of increased DA in AN, Beeler et al. (2021) conclude that increased DA promotes ABA susceptibility by accelerating changes in running wheel activity in response to food restriction.

These findings appear to contradict an earlier study by Foldi, Milton, and Oldfield (2017) that investigated the potential role of increased neuronal activity in the mesolimbic circuit in preventing, and rescuing, weight loss in ABA. Based on evidence from neuroimaging studies suggesting a role for altered reward processing in the development and maintenance of AN (Davis & Woodside, 2002; O'Hara, Campbell, & Schmidt, 2015) the authors chemogenetically activated the mesolimbic pathway, which is primarily dopaminergic (Björklund & Dunnett, 2007). They found that activating this pathway both at the beginning of the ABA phase and, in a different cohort, mid-way through the ABA phase, increased survival thereby preventing the development of ABA and rescuing weight loss. Foldi et al. (2017) found that activation of the mesolimbic pathway at the onset of ABA increased survival by promoting food intake while having no effect on overall running wheel activity. It should be noted that the DREADD expression was not limited to DA neurons and it is therefore possible that the observed effects may have resulted from the activation of midbrain GABA or glutamatergic neurons in the mesolimbic pathway. Furthermore, Foldi et al. (2017) used clozapine-n-oxide (CNO) to drive DREADD activation. As CNO has been shown to be metabolised into the antipsychotic clozapine before it crosses the blood brain barrier (Gomez et al., 2017; Manvich et al., 2018), it is possible that the observed effects were the result of clozapine's actions rather than activation of the mesolimbic pathway per se. Regardless of these limitations, and unlike Beeler et al. (2021) who propose that DA hyperactivity increases ABA susceptibility, Foldi et al. (2017) propose a role for hypoactive mesolimbic DA activity in ABA that can be reversed by increasing DA availability in the NAcc resulting in increased food intake and improved survival rate.

The fact that these two studies appear to suggest opposite directions of change of DA in ABA is representative of the current state of research on DA in AN and illustrates that there is still a lot to understand about the role of DA in different AN symptoms. On one hand, Foldi et al. (2017)'s finding that hypoactivity of the mesolimbic DA system plays a role in ABA is consistent with the view that reward processing, which is dependent on the mesolimbic DA system (Davis & Woodside, 2002), is disrupted in AN and may play a role in maintaining caloric restriction and anhedonia. On the other hand, Foldi et al. (2017)'s findings are in the opposite direction of studies that have examined DA on a more global scale rather than pathway-specific DA (e.g., mesolimbic DA). For instance, the DAT knockdown used in Beeler et al. (2021)'s experiment was global and may have resulted in increased DA in several areas other than the

mesolimbic pathway (e.g., hypothalamus, PFC) which may have resulted in ABA susceptibility. Furthermore, several systemic treatment studies have shown that DA antagonists can promote body weight and survival in ABA (Hillebrand et al., 2005; Klenotich, Ho, McMurray, Server, & Dulawa, 2015; Klenotich et al., 2012; Verhagen, Luijendijk, Hillebrand, & Adan, 2009) thereby suggesting that the effects of ABA can be prevented by decreasing DA signaling. While this may appear to be at odds with the results reported by Foldi et al. (2017), researchers using DA antagonists have done so systemically meaning that the observed effects of DA antagonists on ABA may result from decreased DA signaling in areas other than the mesolimbic pathway. Foldi et al. (2017)'s claims of a hypoactive mesolimbic system playing a role in AN is therefore not necessarily at odds with claims that increased DA activity may also be involved in the illness.

The state and role of DA in AN and ABA is clearly complex and thinking of symptoms of AN as resulting from either DA hyperactivity or hypoactivity is most likely too simplistic. One more probable hypothesis is that the direction of change of DA in AN varies depending on the symptoms and the brain pathway in question. Foldi et al. (2017) found that activating the mesolimbic pathway improved ABA by promoting food intake without effecting running wheel activity. Beeler et al. (2021) found that global DA hyperactivity did not impact food intake but instead accelerated increases in running wheel activity in response to ABA thereby promoting susceptibility. Our own experiment with OLZ resulted in reduced running wheel activity and no effects on food intake. These findings support the hypothesis that, while mesolimbic hypoactivity may interfere with the processing of food reward in ABA, DA hyperactivity elsewhere in the brain may increase susceptibility via increased running wheel activity. Candidate areas include the substantia nigra which is involved in motor control (Crocker, 1997) and the hypothalamus which is involved in the control of feeding and energy expenditure (Timper & Brüning, 2017).

Another potential explanation for the varying results in studies on DA in AN and ABA is the two-stage hypothesis recently proposed by Beeler and Burghardt (2022). Borrowing from the addiction literature, the authors suggest that DA might change throughout the course of AN. In a first stage, rapid weight loss combined with high physical activity is hypothesized to trigger an increase in midbrain DA signaling (via changes in the HPA axis, insulin, leptin, ghrelin, etc.) which facilitates reinforcement learning that fuels further caloric restriction and activity. In response to chronic caloric restriction and physical activity, a second stage begins whereby

decreases in DA result in reduced behavioural plasticity rendering AN-related behaviours more rigid and compulsive (Beeler & Burghardt, 2022). A proposed mechanism put forth by the authors is the upregulation of DA receptor expression and sensitivity resulting in low basal DA. This two-stage DA hypothesis provides a potential explanation as to why DA has been found to be both increased and decreased in AN and in ABA and has important treatment implications. Longitudinal studies in humans with AN as well as in ABA will be needed to provide more support for this hypothesis. It will also be necessary to determine what constitutes “chronic” in the ABA model. Recent efforts by Frintrop et al. (2018) to develop a chronic adaptation of the ABA model will be helpful in this regard.

Contribution of our DA experiments in light of recent publications. In experiment 2.2 (Chapter 4), AMPH-induced locomotor activity was used as a proxy for mesolimbic DA before and after ABA only. As such, we cannot draw any conclusions about mesolimbic DA in ABA susceptibility during ABA. Based on Beeler et al. (2021)’s findings that DA hyperactivity was associated with ABA susceptibility, one might hypothesize that our susceptible rats would show more locomotor activity in response to AMPH had they been tested during acute ABA. AMPH, however, increases locomotor activity via its increased DA signalling in the mesolimbic system (Koob, 1992). Based on Foldi et al. (2017)’s findings suggesting that hypoactivity in the mesolimbic system may play a role in ABA, it is also possible that our ABA susceptible rats would have shown a reduced locomotor response to AMPH compared to resilient rats. Whether susceptible rats have increased or decreased mesolimbic DA signaling compared to resilient rats during ABA, our results suggest that these differences are not present at baseline prior to the onset of ABA. Our results are consistent with Beeler et al. (2021)’s observation that there were no differences in baseline striatal DA between adolescent and adult mice, the former being more susceptible to ABA. Our results also suggest that the higher wheel running activity that we, and others (e.g., Barbarich-Marsteller et al., 2013; Milton et al., 2018; Perez-Leighton et al., 2014; Pjetri et al., 2012), have found to be predictive of ABA susceptibility is not driven by alterations in mesolimbic DA. This finding is consistent with Beeler et al. (2021)’s report that no differences in baseline running wheel activity were observed between DAT knockdown mice and wild-type mice. It is possible that differences in DA in other brain regions between resilient and susceptible rats do play a role in increasing running wheel activity and regulating food intake and candidate areas include the substantia nigra and the hypothalamus as well as the VTA (DA)-

dorsal raphe nucleus (5-HT) circuit (Cai et al., 2022). While there were no indications of baseline mesolimbic DA differences in our rats, it is possible that baseline differences do exist in modulators of DA such as leptin and ghrelin (Adan et al., 2011) which lead to changes in DA in the context of combined extreme caloric restriction and wheel running. Thus, future studies examining DA during ABA as well as DA modulators prior to ABA between resilient and susceptible rats would further the understanding of the role of DA in ABA susceptibility and related underlying mechanisms. Furthermore, in light of the recent publications by Foldi et al. (2017) and Beeler et al. (2021) future studies should aim to investigate the role of DA in ABA susceptibility in specific brain areas and across the full course of ABA.

Reward processing and motivation in ABA susceptibility. Altered reward processing has long been posited to play a role in the development and maintenance of AN (Park, Godier, & Cowdrey, 2014b). Several lines of research discussed throughout this dissertation have provided support for this view. First and foremost, DA functioning in AN has been extensively studied and found to be altered in AN (Broft et al., 2015; Frank et al., 2005; W. H. Kaye et al., 1999; Kontis & Theochari, 2012; Watson et al., 2019) and in ABA (Hillebrand, Van Elburg, et al., 2005; Klenotich et al., 2015; Klenotich et al., 2012; Verhagen, Luijendijk, Hillebrand, et al., 2009; Verhagen, Luijendijk, Korte-Bouws, et al., 2009). Given the well-established role of DA in reward and motivation, these findings point towards a potential role for altered reward in the illness. Secondly, one of the key psychological components of AN is anhedonia which manifests as an inability to derive normal pleasures associated with reward and thus is thought to play a role in patient's ability to withstand severe food restriction (Keating & Rossell, 2014). Lastly, and as discussed in Chapter 6, substance abuse is more prevalent in individuals with eating disorders compared to the general population (Holderness, Brooks-Gunn, & Warren, 1994) and may be indicative of altered reward processing which is a key component of addictive behaviours (Luijten, Schellekens, Kühn, MacHielse, & Sescousse, 2017).

Our findings in the context of recent publications on anhedonia in ABA. In experiment 2.1 (Chapter 4), we assessed for anhedonia-like behaviours in ABA susceptibility by measuring sucrose preference at baseline (i.e., prior to ABA) and were surprised to find no differences between resilient and susceptible rats. These findings, however, were corroborated by a recent publication examining anhedonia in ABA. Using the SPT, Milton et al. (2018) assessed

anhedonia-like behaviour before and during ABA in Sprague Dawley female rats. They found that only a quarter of rats that had preferred sweetened water under baseline conditions developed anhedonia following ABA and, importantly, this was not correlated with ABA susceptibility. More recently, Hurley et al. (2022) examined hedonic responding to sucrose across resilient and susceptible adolescent rats before, during, and after recovery from ABA. The authors scored orofacial responses (e.g., tongue protrusions) in a taste reactivity test to assess “liking” and “disliking”. The authors found no differences in hedonic reactivity at baseline between resilient and susceptible rats which is consistent with our findings and those of Milton et al., (2018) using the SPT. Therefore, evidence thus far suggests that baseline differences in anhedonia do not predict ABA susceptibility. Hurley et al., (2022) did, however, find that susceptible rats, compared to resilient rats, showed reduced “liking” of a palatable tastant while undergoing ABA. This recent finding suggests that, while baseline levels of anhedonia do not predict ABA susceptibility, simultaneous food restriction and running wheel activity during ABA interact to cause changes in reward processing in susceptible rats. Hurley et al. (2022) also observed that susceptible rats, compared to resilient rats, exhibited lower astrocyte density in the mPFC following recovery from ABA. This finding is consistent with recent reports of lower astrocyte density in rodents maintained on a chronic ABA model (Frintrop et al., 2018) as well as reports from clinical studies indicating cortical thinning in patients acutely ill with AN (King et al., 2015; Seitz et al., 2014). More research is needed to determine how, if at all, decreased astrocyte density in the mPFC relates to altered hedonic responding during ABA in susceptible rats.

The discrepancy in anhedonia results depending on the assay used emphasizes the importance of including more than one measure in future studies and raises the question about what these tests are assessing. Anhedonia reflects the disruption of a single facet in the complex concept of reward processing which includes pleasure expectation, reward evaluation, gauging of required effort, planning, and decision-making (Scheggi et al., 2018). Disruption of any of these aspects may lead to behaviours that could be interpreted as anhedonia (Scheggi et al., 2018). Both the SPT used by us and Milton et al. (2018) and the taste reactivity taste used by Hurley et al. (2022) are thought to measure the “liking” component of reward-related behaviour. Milton et al. (2018) propose that anhedonia in ABA may not present itself as a difference in “liking”, which was intact in their experiment, but rather by a difference in the “wanting” or motivational

aspect of reward (Berridge, 2009) which they argue is not captured by the SPT because no extra effort is required to gain access to the sweetened water. This view is consistent with findings from studies of taste reward in AN that reveal differences in “wanting” between patients and healthy control participants despite evidence that “liking” remains intact (reviewed in Keating, Tilbrook, Rossell, Enticott, & Fitzgerald, 2012). Hurley et al. (2022)’s findings, however, would suggest that susceptible and resilient rats do differ when hedonic responding is isolated using the taste reactivity test rather than the SPT which, they argue, may be influenced by appetite. An additional issue with the SPT is the lack of variability which can complicate the detection of preference differences. This was an issue in our experiment whereby preference was close to 100% for all rats. The question of anhedonia at different stages of the ABA protocol will need to be further investigated given these inconsistencies and methodological considerations. It would be pertinent to assess for differences in hedonic responding between resilient and susceptible rats using both the SPT and the taste reactivity test before, during, and following recovery from ABA. Furthermore, research is needed on other components of anhedonia, including motivation, in ABA. One way of exploring both the “liking” and “wanting” components of reward-related behaviour is by using drug self-administration protocols. Our experiment in Chapter 6 is the first, and currently the only, study examining drug self-administration in the ABA model.

Drug self-administration in ABA. In Chapter 6, we designed a protocol allowing us to examine cocaine self-administration in rats with a history of two bouts of ABA. We made use of the reinstatement model which enabled us to examine drug-taking behaviours as well as relapse. Furthermore, using principles of behavioural economics, we were able to compare rats’ motivation to work for cocaine. Our results indicated that rats that had experienced ABA, compared to control rats, showed more motivation to work for cocaine (i.e., P_{Max}) and were more resistant to extinction. There were no differences in Q_0 (i.e., preferred consumption level when price was negligible), suggesting that ABA led to changes in “wanting” but not in “liking.” These results are discussed in detail in Chapter 6. Taken together with our earlier findings from the SPT, our results are consistent with the view that the “liking” aspect of reward may be intact in ABA but that “wanting” may be impacted. In our cocaine self-administration experiment, we assessed drug-taking behaviours following the experience of ABA and we therefore cannot determine whether differences in reward-related behaviours were present at baseline. Our results do suggest that the combination of caloric restriction and excessive activity resulting in rapid

weight loss leads to changes in reward processing that may play a role in the maintenance of the disorder as well as the heightened risk for substance abuse observed in the AN population.

While speculative, one potential explanation of the mechanism that occurred during ABA and resulted in later in altered reward processing is the first stage of the two-stage DA hypothesis offered by Beeler and Burghardt (2022) described above. Beeler and Burghardt (2022) suggest that, in the first stage of the disorder, caloric restriction combined with excessive exercise triggers an increase in midbrain DA signalling which reinforces further caloric restriction and excessive exercise. The authors propose that these changes in DA may be mediated by stress-induced activation of the HPA axis, changes in insulin sensitivity, and/or changes in leptin and ghrelin concentrations. From this perspective, it is possible that the experience of ABA in our rats resulted in increased midbrain DA signaling persisting even after recovery from ABA and that this change contributed to the increase motivation to work for cocaine that we observed in ABA rats.

An alternative explanation for the increased motivation to work for cocaine that we observed in rats that had experienced ABA also comes from the addiction literature and is the concept of reward deficiency. The reward deficiency syndrome was first proposed by Blum et al. (2000) to provide a clinically relevant term for conditions involving deficits in mesocorticolimbic DA function. The proposal is that substance use is the consequence of a low functioning mesolimbic DA system which predisposes an individual to seek psychoactive substances and behaviours to release DA in the reward circuit to overcome DA deficits (Blum et al., 2000). A more contemporary view of this hypothesis is that a deficit state does not necessarily induce reward-seeking behaviours but can rather produce a contrast effect, increasing the drawing power of rewards once the reward has been experienced (Leyton, 2014). In the case of our cocaine experiment, it is possible that the experience of ABA led to persisting deficits in the mesolimbic DA circuit which is consistent with the two-stage DA model of AN proposed by Beeler and Burghardt (2022). This hypodopaminergic state may result in rats requiring a greater amount of cocaine to experience a similar level of reward compared to control rats. Interpretation of our results with the reward deficiency hypothesis in mind, suggests that individuals with AN experience hypodopaminergic activity, either due to a predisposition or as a result of the

combination of self-starvation and excessive exercise, which increases their risk of becoming addicted to sampled drugs of abuse.

A more relevant question, and certainly our main area of interest, was the question of whether differences exist in drug-taking behaviours between resilient and susceptible rats with a history of ABA. As discussed in Chapter 6, our ability to draw conclusions about potential trait differences was limited by a very small sample size. Our preliminary findings were nonetheless interesting and contrary to our hypothesis. While we found that a history of ABA led to increased motivation to work for cocaine, we found that rats with the most severe experience of ABA (i.e., susceptible rats) were less motivated to work for cocaine compared to the rats that had a less severe experience of ABA (i.e., resilient rats). This result is difficult to interpret given the preliminary nature of this dataset and the absence of any other publications on the topic. Although quite speculative, these results may indicate that the relationship between ABA susceptibility and motivation to work for a reward is nonlinear. Instead, it is possible that the relationship between ABA severity and motivation for reward is that of an inverted-U curve whereby susceptible rats' experience of ABA renders them so anhedonic that they lack the motivation to "put in the work" required to compensate for the presence of a reward deficit. Although entirely speculative at this stage, this hypothesis can be supported by the human literature showing that, while substance abuse is more prevalent in individuals with eating disorders compared to healthy control participants (Holderness et al., 1994), rates tend to be higher in individuals with BN and AN-BP compared to individuals with AN-R who accomplish excessively low weight through food restriction and exercise and are more likely to be described as anhedonic (rather than purging; Herzog, Nussbaum, & Marmor, 1996; Root et al., 2010).

Executive functioning in ABA and link to susceptibility. While not official criteria for diagnosis, disruptions in executive functioning have consistently been reported in AN and are thought to play a fundamental role in the development and maintenance of the disorder (Galimberti et al., 2013, 2012; Holliday, Tchanturia, & Landau, 2014; Lindner et al., 2014; McAnarney et al., 2011). Executive functions, including cognitive flexibility and response inhibition rely on the PFC which has widespread connections with subcortical regions (Gabbott, Warner, Jays, Salway, & Busby, 2005). Of particular interest to AN and ABA is the interaction between the mPFC and the NAcc shell which is involved in the top-down control of reward-

based feeding (Berridge, 2009) and cognitive flexibility (Block et al., 2007; Piao et al., 2017). Milton et al. (2020) propose that disruption in this frontostriatal circuit is involved in the excessive control over feeding in patients with AN. More specifically, decreased activity in mesolimbic “reward” circuitry and increased activity in prefrontal “control” circuitry are thought to interact and override homeostatic requirements for energy balance, resulting in severe weight loss in AN (Milton et al., 2020). It is only in recent years that researchers have begun to examine cognitive functioning in rodents using the ABA model. Three experiments from the present dissertation add to the small pool of studies in this area. Each of these studies are discussed thoroughly in their respective chapters but will be revisited here briefly in the context of the frontostriatal hypothesis.

Our efforts to assess for differences in neural activity between resilient and susceptible rats provided support for the view of altered frontostriatal circuitry in ABA. We found that susceptible rats, compared to resilient rats, showed a trend for increased neural activity (as measured by c-fos immunohistochemistry) in the PFC and reduced activity in the NAcc shell. These findings are in line with a recent study by Milton et al. (2020) in which chemogenetic inhibition of the mPFC-NAcc shell pathway was found to reduce the development of ABA and improve cognitive flexibility.

We assessed cognitive functioning in ABA in two studies. First, in experiment 1.6 (Chapter 3), we assessed the effect of ABA on set-shifting ability. At the time of this experiment, no other studies had been published on cognitive flexibility in ABA. We compared rats exposed to ABA to control sedentary rats and found no differences in set-shifting. As explored in the experiment discussion, however, this initially surprising finding may be consistent with a recent publication suggesting that reversal learning, a component of cognitive flexibility, is impaired in ABA while set-shifting seems to remain intact (Allen et al., 2017). At the time of this experiment, we had not yet begun to examine differences between resilient and susceptible rats and had thus designed the experiment to examine the effect of ABA on cognitive flexibility rather than assess for a link with ABA susceptibility. Future studies examining cognitive flexibility relative to ABA susceptibility will be necessary and should include measures of both set-shifting ability and reversal learning.

In addition to cognitive flexibility which is the cognitive function that has been most researched in AN and now ABA, we also examined response inhibition in experiment 3.2 (Chapter 5). Unlike our cognitive flexibility experiment, this study was designed precisely to assess for trait differences in impulsivity. We did not observe any differences between resilient and susceptible rats suggesting that response inhibition, as measured by the Go/No-Go task, may not play a role in ABA susceptibility. To the best of our knowledge, there have been no other studies examining impulsivity in the context of ABA and more studies are necessary.

Taken together, our findings provide more questions than answers regarding the contribution of the frontostriatal circuit and altered executive control in ABA. While our results using Fos immunohistochemistry are consistent with the view that an overactive PFC might be involved in the rigid behaviours observed in AN, it is unclear, based on our results, if cognitive impairment is present in the ABA model and whether or not it is associated with susceptibility. Our experiments have demonstrated that it is possible to use cognitive tasks, often requiring significant training periods, in the context of ABA. While neurocognitive functioning has been repeatedly assessed in AN, a thorough neurocognitive assessment in ABA is missing. Lamanna et al. (2019) propose a set of relevant neurocognitive assays that should be performed in ABA and include measures of cognitive flexibility and impulsivity as well as working memory and decision-making. Given Beeler and Burghardt (2022)'s proposal that DA signalling and behavioural flexibility change across the course of illness, it will be important to assess these behaviours in ABA early in the development of ABA as well as after chronic exposure to ABA.

Methodological considerations

Since its inception in the 60s, the ABA model has been employed using various protocols. Consequently, several influencing factors have been identified such as pre-exposure to the wheel, ambient temperature, feeding schedule, diet, sex, strain, etc. A discussion of the many factors influencing ABA development is beyond the focus of this dissertation but is available in a thorough review by Schalla and Stengel (2019). Nonetheless, the development of the ABA model in our laboratory required many trials and variations that are worth grouping and discussing briefly here for future studies.

Sex differences. It has been previously suggested that, when exposed to ABA, female rats eat more than male rats (Doerries et al., 1991). Furthermore, the early observations that,

under conditions of severe food restriction, active male rats ate less than sedentary rats, has never been replicated in female rats. Although none of the experiments presented in this dissertation included direct comparisons between males and females, comparisons across the experiments in Chapter 3 provide some important observations. Interestingly, we found that the introduction of the running wheel under *ad libitum* feeding conditions resulted in a temporary feeding suppression (i.e., wheel-induced feeding suppression) accompanied by weight loss in male rats (experiment 1.3). Females, however, increased their food intake when the wheel was introduced, allowing them to maintain their body weight. As such, while the wheel-induced feeding suppression observed in male rats is undoubtedly relevant for studying the relation between food intake and activity, it appears to fall short as a model of AN-like symptoms if it cannot be replicated in female rats. Sex differences in food intake in response to running wheel activity disappeared under conditions of restricted feeding. Unlike the early ABA reports that active male rats ate less than sedentary rats when food restricted (Routtenberg & Kuznesof, 1967), we found that active rats ate the same amount as sedentary rats despite their higher energy expenditure and that this was true for both males and females. In both sexes, the failure to increase their energy intake to compensate for their increased energy expenditure resulted in accelerated weight loss.

Female rats have repeatedly been shown to run more than male rats. Regardless of this higher activity in female rats, Boakes, Mills, and Single (1999) suggested that food restriction and accompanying weight loss led to an increase in running wheel activity in males only. Our findings were consistent with the literature in that females were generally more active than male rats. For instance, when comparing wheel activity between experiments 1.2 and 1.4 (both experiments used a similar timeline and a 60-min feeding window), females ran nearly 2x more than males during the wheel habituation phases (under sated conditions) and approximately 70% more than males during the food restricted phases. Unlike the results reported by Boakes et al. (1999), however, we found that the onset of food restriction resulted in increased running wheel activity in both males and females. Furthermore, we found that the effect was larger in females ($d = 2.08$, experiment 1.4) than in males ($d = 1.06$, experiment 1.2). We also found that weight loss in response to simultaneous wheel activity and food restriction was accelerated in females compared to males. For instance, male rats in experiment 1.2 that were exposed to a 60 min/day food restriction while active had lost, on average, 25% of their initial body weight after 7 days while female rats from experiment 1.4 exposed to the same feeding schedule reached this

criterion after 5 days. Thus, our findings suggest that, while ABA does develop in both male and female rats, the effect is more robust in females, lending more support to the use of ABA as a model of AN, a predominately female disorder.

Duration of feeding window. The duration of the feeding window used in the ABA model has varied greatly across studies ranging from 30 min to 3 hrs (reviewed in Schalla & Stengel, 2019) with the most common duration being 60 min and 90 min. In our first experiment using female rats (experiment 1.4), we used a 60 min feeding window as this duration had proved effective in developing ABA in male rats. As reported above, however, we found that female, compared to males, lost weight more rapidly and reached starvation criterion after only 5 days under this feeding schedule. In an effort to prolong the ABA phase, we subsequently used a 90 min feeding window (experiment 1.5) and found that rats began to reach starvation criterion on the 9th day of this phase, as opposed to the 5th day when a 60 min feeding window was used. However, we also found that rats were less active than those in experiment 1.4 (60 min feeding window) and did not show significant food restriction-induced hyperactivity. Our results therefore suggest that, in female rats, the 90 min feeding window may not be severe enough to produce the hyperactivity effect that is so crucial to ABA.

One versus two bouts of ABA. The present dissertation includes experiments in which rats were exposed to either one bout of ABA or two bouts of ABA separated by a recovery period. In some cases, the two-bout procedure was selected in the context of manipulation studies whereby the first bout was used to identify resilient and susceptible rats and the second bout was used to examine the effect of a given manipulation on ABA susceptibility. In other case, the two-bout model was used simply to mimic the relapsing nature of AN. In addition, the two-bout model has been reported to exacerbate individual differences in C57/BL6 mice, allowing for the study of resilience and susceptibility (Chowdhury et al., 2013). As individual differences became the focus of this dissertation, we hoped to amplify them using two bouts of ABA. Repeatedly, however, we found that rats appeared to adjust their behaviour during the second bout of ABA compared to the first, thereby reducing the gap between resilient and susceptible rats. Susceptible rats decreased their running wheel activity from the first to second bout, increased their food intake and appeared to lose less weight during the second ABA compared to the first. These results are somewhat inconsistent with reports in C57/BL6 mice.

Similar to our findings, Chowdhury et al., (2013) do report prolonged survival during a second bout of ABA suggesting behavioural adaptation, but the distinction between resilient and susceptible animals is accentuated as opposed to the reduction of trait differences observed in the present experiment. One possible explanation for this discrepancy is the age of the animals. Chowdhury et al. (2013) used adolescent mice that were approximately at PND51 at the start of the second bout of ABA. In the present dissertation, rats were well into adulthood by the start of the second bout of ABA with their age ranging from PND110 to 150. Based on our findings, it therefore appears that one bout of ABA, rather than two, is preferable to study individual differences in adult female rats. Age has previously been shown to effect ABA development whereby ABA develops more rapidly and reliably in younger rats (Frintrop et al., 2018). This is also the case in mice where by adolescent mice have been shown to be more susceptible to ABA than adult mice (Beeler et al., 2021). Researchers interested in reproducing the relapsing nature of AN by using several bouts of ABA in female rats may have more success doing so with adolescent rats.

Clinical Implications

All experiments presented in this dissertation were designed and conducted with one population in mind: individuals suffering from AN. While these studies are fundamental and preclinical in nature, we would be remiss not to discuss the implications of our findings for the clinical population. As can be seen from the above discussion, the experiments presented here led to more questions than answers and will require further investigation and replication before confident conclusions can be drawn about AN. Nonetheless, the identification of ABA susceptible rats who are more hyperactive, both at baseline and during ABA, has implications for the assessment, treatment, and diagnosis of AN.

The importance of assessing hyperactivity in AN, beyond its function as a weight loss strategy. Excessive exercise, though not a necessary criterion for the diagnosis of AN, is a common feature of the disorder (Davis et al., 1997; Rizk et al., 2015). Clinically, physical activity in AN is typically thought of as a means for weight loss and proposed assessment guidelines recommend the assessment of compensatory behaviours such as exercise (Fairburn et al., 2003). What can be missed in our current assessment of AN is the presence of excessive physical activity that may serve other functions, in addition to the attempt to burn calories, and

play an important role in maintenance and severity of symptoms. Using the ABA model, we found a subset of rats that were more susceptible to ABA whereby they reached starvation criterion at a faster rate. A consistent finding across our studies was that these susceptible rats were more active on the running wheel during ABA, but also prior to food restriction. While our understanding of why hyperactivity occurs in ABA is still as its infancy, we can be certain that susceptible rats do not run more as a result of a greater concern with body shape and weight. Instead, an interaction between running wheel activity and food restriction occurs in these more susceptible rats plunging them into a downward cycle preventing adaptation and resulting in rapid weight loss. If we extend this to humans, this suggests that baseline levels of hyperactivity, prior to the onset of illness, may be a risk factor for AN and serve as a predictor of symptom severity. As such, initial assessments should include a timeline of a patient's hyperactivity as well as a thorough understanding of its function at different times across the course of illness. Early identification of individuals with AN who may have a similar susceptibility can have important implications for treatment.

To encourage or discourage exercise, that is the question. Given that excessive exercise in AN contributes to emancipation and interferes with recovery, practitioners often need to work with patients and their caregivers, when appropriate, to limit or stop exercise completely during treatment (Calogero & Pedrotty, 2004). When patient compliance is achieved, this approach can be quite successful. The reality, however, is that patients are often reluctant to give up exercise which can cause ruptures in the therapeutic alliance (Mathisen et al., 2018). Furthermore, engagement in exercise has many known physical and mental health benefits and has been associated with improved quality of life and a reduction in comorbid depression and anxiety in the treatment of AN (Cook et al., 2016; Dittmer et al., 2018; Rizk et al., 2015). For these reasons, Alanah Dobinson (exercise physiologist), Marita Cooper (clinical psychologist), and Danika Quesnel (kinesiologist) have recently teamed up to create the Safe Exercise at Every Stage guidelines to help treatment teams safely manage exercise during ED treatment (Dobinson, Cooper, & Quesnel, 2021). If we extrapolate our findings with resilient and susceptible rats to humans, it is possible that there are pre-existing trait differences that may make exercise during treatment appropriate for some and contraindicated for others. This is consistent with the finding that female mice that showed the resilient trait gained more weight under food restriction when allowed access to the running wheel compared to mice that had no access to the running wheel

(Beeler et al., 2021). A thorough assessment of patient's levels of hyperactivity and its function prior to illness onset may help identify individuals who are more at risk to determine whether or not engagement in exercise is indicated.

Taking the time to understand a person's history of hyperactivity and the function that it serves can also have implications for treatment beyond inclusion or exclusion of exercise. In a large number of cases, exercise does serve the purpose of weight loss or compensation for perceived overeating (Dittmer et al., 2018). In these cases, exposure is used as an intervention whereby patients are gradually brought to expose themselves to feared foods and compensatory exercise is prevented, not unlike exposure and response prevention in the treatment of OCD (Fairburn et al., 2003). Such an intervention, when relevant, helps patients habituate to anxiety and challenge their beliefs. Our research suggests that, in a subset of individuals, physical activity or hyperactivity may be linked to traits and may be maintained for reasons other than (or in addition to) the fear of weight gain. For instance, our findings and those of other researchers (e.g. Wable et al., 2015) suggest that running wheel activity during ABA may be associated with anxiety. Some more anxious individuals with AN may engage in physical activity to regulate anxiety and would benefit from targeted interventions aimed at helping them develop distress tolerance and emotion regulation skills to replace physical activity. Given that delayed gastric emptying is a common physical symptom of AN (Rigaud et al., 1988; Stacher, 2003; Szmukler, Young, Lichtenstein, & Andrews, 1990), some patients may engage in physical activity to relieve this post-meal gastric discomfort. This is supported by our preliminary findings ABA susceptibility may be associated with more PPA. In such cases, individuals may benefit from exposures centered around tolerance of gastric discomfort.

Rethinking energy intake in diagnosis. One of the earliest findings in this dissertation was that, unlike what one might expect in a model of AN, ABA susceptible rats did not necessarily eat less than resilient rats. Instead, susceptible rats typically ate the same amount or more than resilient rats. Their food intake, however, was not sufficient to compensate for their increased energy expenditure on the running wheel. This is an important reminder for researchers and clinicians that caloric intake cannot be considered in a vacuum and that what may not be restrictive for one person may be dangerously restrictive for another. This point is captured in the first diagnostic criterion for AN in the DSM-5: "Restriction of energy intake relative to

requirements, leading to a significantly low body weight in the context of age, sex, developmental trajectory, and physical health.” Nonetheless, AN is often missed in highly active populations who may not be obviously restricting and underweight such as elite athletes (Byrne & McLean, 2002; Sundgot-Borgen, 1993). Recently in popular media, athletes have increasingly been coming forward about their mental health struggles including those who have struggled with eating disorders. Indeed, athletes are at higher risk of developing AN (Arcelus, Witcomb, & Mitchell, 2014; Byrne & McLean, 2002; Sundgot-Borgen, 1993; Sundgot-Borgen & Torstveit, 2004), especially those in the performing arts, sports involving weight categories, and endurance sports (Currie, 2010). Symptoms of AN or other eating disorders are often missed in these populations as they are assumed to be “normal” athlete behaviours and beliefs (Currie, 2010). This complicates the recognition of the eating disorder by the training team, support systems, the person themselves, and even health professionals. Results from the ABA model emphasize the role of activity in symptom severity and stress the importance of always considering caloric intake in the context of energy expenditure in AN.

Conclusion

The studies presented in this dissertation provide insights into the ABA model and, more specifically, ABA susceptibility. In summary, there is important individual variability in rats’ response to the ABA procedure. While some rats can survive under this protocol by either adjusting their food intake or running wheel activity, a subset of rats show significantly higher wheel activity and fail to adjust their food intake resulting in a negative energy balance and rapid weight loss. By comparing these resilient and susceptible rats, we found important differences in baseline running wheel activity as well as in performance on the forced swim task. We also observed statistical trends for differences in neural activity in the PFC and NAcc as well as differential responding to cocaine self-administration.

Overall, our results emphasize the importance of focusing on individual differences in future ABA studies. We have also shown that it is possible to use the ABA procedure in combination with more elaborate behavioural paradigms allowing for the assessment of cognitive functioning and addiction-like behaviours. Moving forward, we hope that the study of ABA resilience and susceptibility will further our understanding of what makes certain

individuals more at risk of developing AN and that this knowledge will inform prevention, assessment, and treatment.

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APPENDIX 1: THE EFFECT OF PRE-EXPOSURE TO THE FEEDING SCHEDULE ON THE DEVELOPMENT OF ABA

Since the original Routtenberg and Kuznosof (1967) experiments, many researchers have sought to understand the mechanism underlying ABA development. The first major theory that emerged is the *suppression theory* which posits that ABA occurs as a direct result of the suppression in the reinforcing value of food when an animal engages in high levels of physical activity and the increased reinforcing value of wheel running during food restriction (Pierce, Epling, & Boer, 1986). In 1997, Dwyer and Boakes proposed the *adaptation theory* as an alternative explanation to the suppression theory. In their publication, the authors tested the effect of a pre-exposure to a 90-min per day feeding schedule by comparing a preadapted group and a nonadapted group. They found that the preadapted rats began to recover their weight across the 7 days of the ABA procedure and that none of them reached the removal criterion (75% of their *ad libitum* weight over 2 consecutive days) while none of the nonadapted rats recovered and five out of eight rats reached the removal criterion. Based on these findings, Dwyer and Boakes (1997) suggested that ABA occurs due to a failure to adapt to a restricted feeding schedule when the opportunity to exercise is available and thus questioned the utility of ABA as it relates to human AN. Shortly after, Lett, Grant, Smith, and Koh (2001) provided a rebuttal against the adaptation theory by examining the effect of pre-exposure to the feeding schedule on the development of ABA. In their experiment, all rats underwent a preadaptation phase in which they had access to food for 90 min a day while remaining sedentary until their body weight stabilized (15 days). After this phase, experimental rats underwent the ABA procedure in which they continued to be food restricted while given access to a running wheel and the control rats did not have access to a wheel but also continued to be food restricted. Lett et al. (2001) found that, during the ABA phase, experimental rats ate significantly less food and lost significantly more weight than the control rats, thus concluding that pre-exposure to the restricted feeding schedule before exposure to the ABA procedure is not effective in eliminating ABA. Instead, they suggested that wheel activity suppresses feeding (suppression theory) which begins the vicious cycle of ABA and that preadaptation to the feeding schedule is a modulating variable that can slow the ABA effect but not eliminate it.

Pre-exposure to the feeding schedule is typically omitted from current ABA protocols. Based on the early reports by Routtenberg and Kuznesof (1967) that a daily feeding window of 60 min is optimal to obtain a robust ABA effect in experimental rats while allowing sedentary control rats to maintain their body weight, the majority of contemporary ABA experiments utilize this feeding duration. There is, however, no evidence to suggest that the robust ABA effect observed is not merely the consequence of a failure to adapt to this more severe restricted feeding schedule as all experiments testing the effects of pre-exposure to the feeding schedule on ABA have utilized a 90 min/day feeding window. Furthermore, all experiments examining the effect of pre-exposure to the feeding schedule on ABA have been carried out in albino rats. To the best of our knowledge, the ABA procedure has not been tested in Long-Evans male rats and there is no research examining the impact of pre-exposure to the feeding schedule on the development of ABA in this specific strain.

In this first pilot study, we aimed to test whether ABA would develop in Long-Evans male rats pre-exposed to a feeding schedule of 60 min/day. During the restricted feeding-sedentary phase (pre-exposure), we expected that rats would adapt and stabilize their body weight. During the restricted feeding-active phase, we expected that food intake would eventually stabilize at pre-wheel levels but that rats would continue to lose weight and reach removal criterion, despite pre-exposure to the feeding schedule.

Method

Subjects

Male Long-Evans rats ($n = 6$; 325-350 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec) and housed in a colony room on a 12:12 hr reverse light/dark cycle. Upon arrival, rats were initially pair-housed in plastic shoebox cages and were then separated into individual shoebox cages (experimental day 1). On day 4, rats were transferred to the laboratory where they were permanently housed in running wheel cages inside sound-attenuating boxes until the end of the experiment. Body weight (g), food intake (g), and water intake (g) was monitored daily at ZT 11-12. According to the survival guidelines offered by Routtenberg and Kuznesof (1967), rats were removed from the experiment once they reached survival criteria of daily food intake below 1 g.

Apparatus

Running Wheel Cages. See “General Methodology” section.

Procedure

The detailed timeline is depicted in Figure A1.1.

Acclimation phase (days 1-4). Rats were individually housed in plastic shoebox cages for 4 days in the Animal Care Facility, allowing them time to acclimate to the new environment. This period also allowed for baseline daily measures of body weight, food intake, and water intake.

Restricted feeding-sedentary phase (days 5-15). During these 11 days, rats were housed in running wheel cages. The running wheels were locked throughout this entire phase and rats were thus sedentary. On the first day of this phase, food was removed at ZT 13. On the following days, rats had access to food for 1 hr/day between ZT 12-13. The purpose of this phase was to allow the rats a period, prior to introducing the running wheel, during which they could habituate to the new feeding schedule. Furthermore, this phase provided baseline measures of

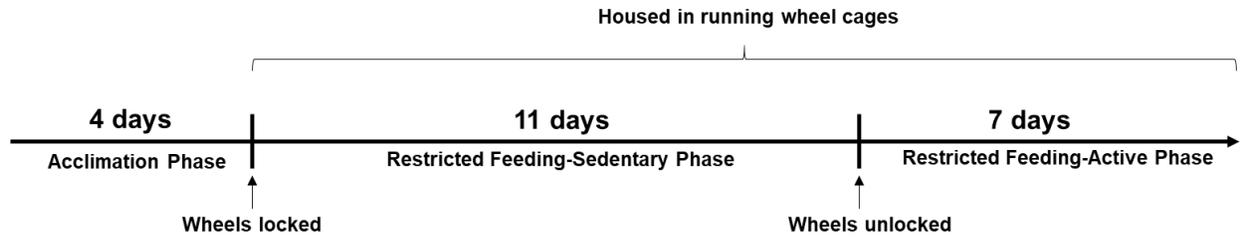


Figure A1.1. Timeline of supplemental experiment 1.

food intake and weight loss under a restricted feeding schedule which could be compared to these same variables upon introduction of the running wheel.

Restricted feeding-active phase (days 16-22). Following 11 days of restricted feeding, the running wheels were unlocked and rats had continuous access to their running wheels. The restricted feeding schedule continued as described above and the experiment was terminated after 7 days of this phase.

Statistical Analysis

Food intake. To assess for changes in food intake from the acclimation phase to the restricted feeding-sedentary phase, a within-subjects *t*-test was used comparing food intake averaged across the 4 days of the acclimation phase with food intake averaged across the first 4 days of the restricted feeding-sedentary phase. A one-way repeated-measures ANOVA was then used to compare food intake across the 11 days of the restricted feeding-sedentary phase. To assess whether food intake has stabilized by the end of the restricted feeding-sedentary phase, a one-way repeated-measures ANOVA was used on the last 4 days of this phase. A one-way repeated-measured ANOVA was also used on the first 4 days of the restricted feeding-active phase to assess for wheel induced feeding suppression. Finally, a within-subjects *t*-test was used to compare food intake averaged across the last 4 days of the restricted feeding-sedentary phase to the food intake averaged across the first 4 days of the restricted feeding-active phase.

Body weight. To characterize the change in body weight from the acclimation phase to the restricted feeding-sedentary phase, a within-subjects *t*-test was used comparing body weight on the last day of the acclimation phase and the first day of the restricted feeding-sedentary phase. A one-way repeated-measures ANOVA was used to compare body weight across the 11 days of the restricted feeding-sedentary phase. A within-subjects *t*-test was then used to compare body weight averaged across the last 4 days of the restricted feeding-sedentary phase to the body weight averaged across the first 4 days of the restricted feeding-active phase. Finally, a within-subjects *t*-test was used comparing change in body weight averaged across the last 4 days of the restricted feeding-sedentary phase to the change in body weight averaged across the first 4 days of the restricted feeding-active phase.

Running wheel activity. A one-way repeated-measures ANOVA was used to characterize running wheel activity across the first 4 days of the restricted feeding-active phase.

Results

Data Integrity

As no outliers (z -score ≥ 2.00) were identified across all variables of interest, all 6 rats were included in the analyses. One rat was removed from the experiment on day 20 (5th day of the restricted feeding-active phase) due to severe starvation symptoms. As such, analyses of food intake, body weight, and activity during the restricted feeding-active phase were limited to the first 4 days only.

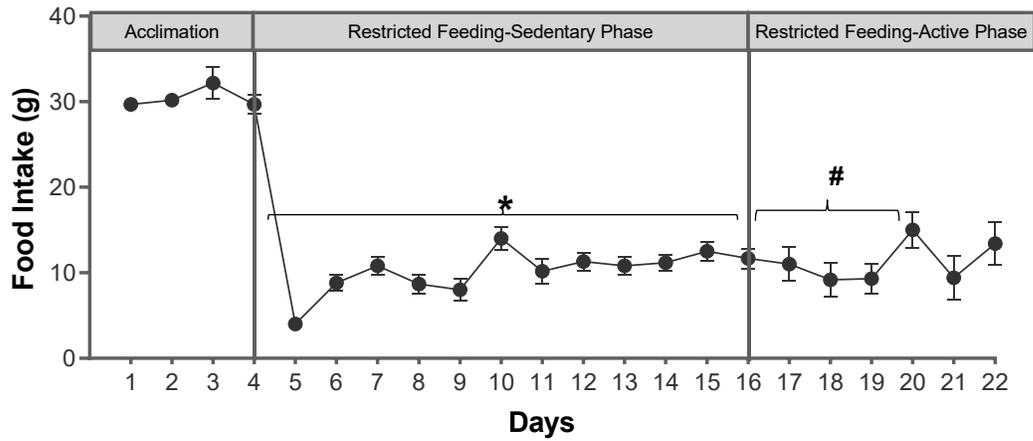
Food intake

As can be seen in Figure A1.2A, initiation of the restricted feeding-sedentary phase resulted in a sharp decrease in food intake. Indeed, the onset of the restricted feeding-sedentary phase resulted in a statistically significant decrease in food intake compared to the acclimation phase ($t(5) = 30.50, p < .001, d = 12.45$, Figure A1.2B). Food intake increased across the 11 days of the restricted feeding-sedentary phase indicating that rats learned the feeding schedule ($F(10,50) = 7.08, p < .001, \eta_p^2 = .59$; Figure 3.2A). Across the last 4 days of the restricted feeding-sedentary phase, food intake remained stable ($F(3,15) = 1.22, p = .337, \eta_p^2 = .20$; Figure 3.2A). As can be seen in Figure 3.2A, introduction of the running wheel (day 16) resulted in a gradual decrease in food intake over the first 4 days of the restricted feeding-active phase, though this decrease did not reach statistical significance ($F(3, 15) = 2.81, p = .075, \eta_p^2 = .36$). The start of the restricted feeding-active phase did not result in a change in food intake from that of the restricted feeding-sedentary phase ($t(5) = 0.73, p = .249, d = 0.31$; Figure A1.2C).

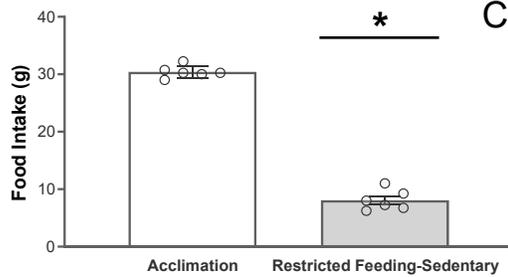
Body weight

As seen in Figure A1.3A, initiation of the restricted feeding-sedentary phase (day 5) resulted in rapid weight loss. Body weight on the first day of the restricted feeding-sedentary phase was significantly lower than on the last day of the acclimation phase ($t(5) = 4.97, p = .002, d = 2.03$, Figure A1.3B). Rats gradually and steadily lost weight across the 11 days of the restricted feeding-sedentary phase ($F(10,50) = 55.23, p < .001, \eta_p^2 = .92$; Figure A1.3A). As can

A



B



C

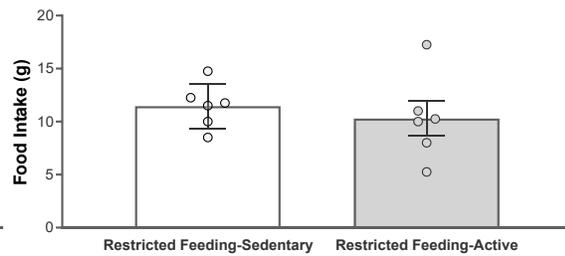


Figure A1.2. The effect of running wheel activity on food intake under a restricted-feeding schedule. (A) Daily food intake across experimental days, * $p < .001$, main effect of *days*; # $p = .075$, statistical trend for *days*. (B) Average daily food intake across the 4 days of the acclimation phase compared to the first 4 days of the restricted feeding-sedentary phase, * $p < .001$. (C) Average daily food intake across the last 4 days of the restricted feeding-sedentary phase compared to the first 4 days of the restricted feeding-active phase.

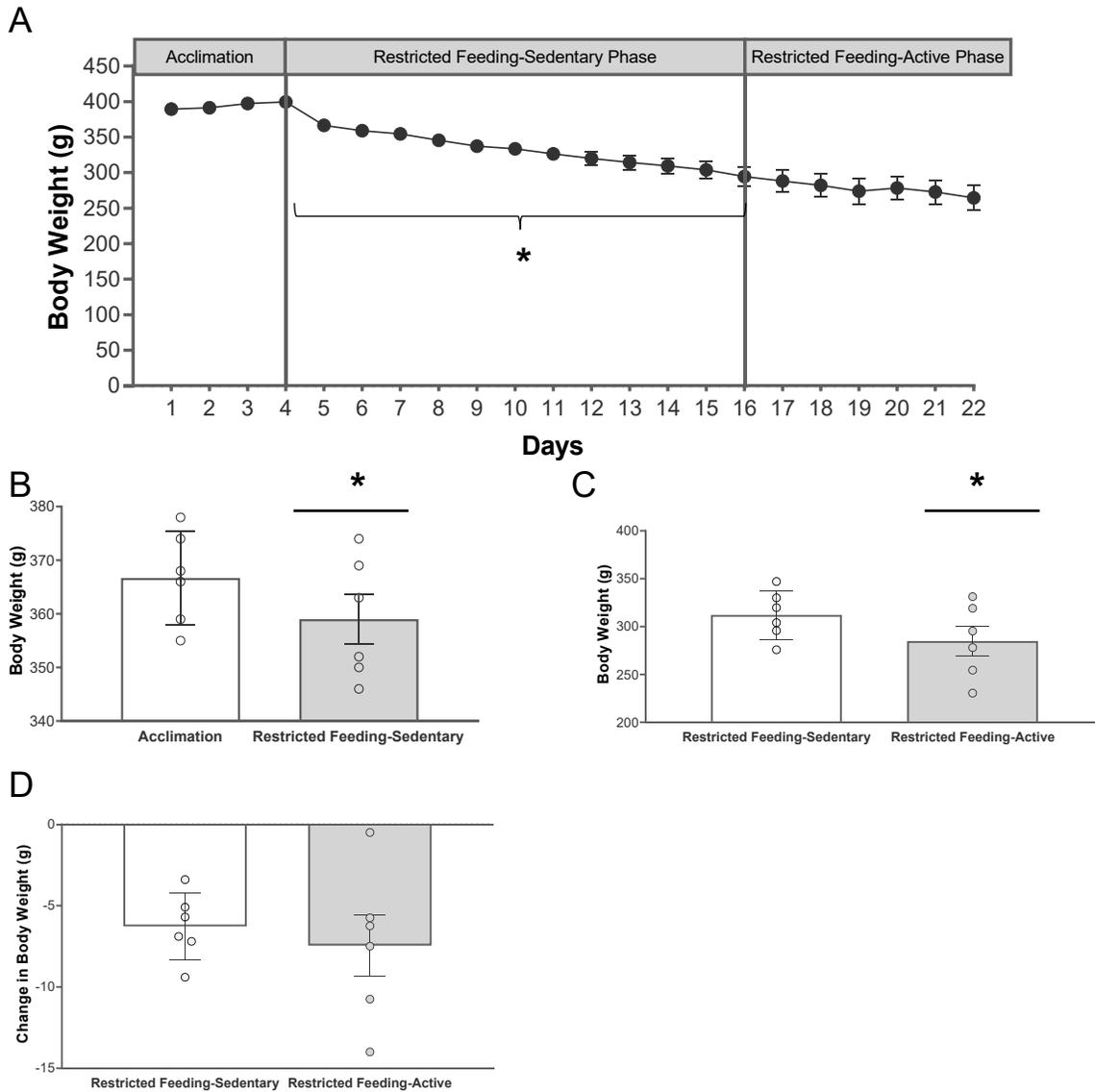


Figure A1.3. The effect of running wheel activity on body weight under a restricted-feeding schedule. (A) Body weight across experimental days, * $p < .001$. (B) Body weight on the last day of the acclimation phase compared to the first day of the restricted feeding-sedentary phase, * $p = .002$. (C) Average body weight across the last 4 days of the restricted feeding-sedentary phase compared to the first 4 days of the restricted feeding-active phase, * $p = .002$. (D) Average change in body weight across the last 4 days of the restricted feeding-sedentary phase compared to the first 4 days of the restricted feeding-active phase.

be seen in Figure A1.3A, rats continued to lose weight when the running wheel was introduced on day 16. The introduction of the running wheel resulted in a significant decrease in body weight ($t(5) = 4.91, p = .002, d = 2.01$, Figure A1.3C). Importantly, however, there was no difference in the rate of weight loss (change in body weight) between the restricted feeding-sedentary phase and the restricted feeding-active phase ($t(5) = 1.09, p = .163, d = 0.44$, Figure A1.3D). By the 4th day of the restricted feeding-active phase, rats weighed an average of 67.82% ($SEM = 4.42$) of their initial body weight.

Running wheel activity

As can be seen in Figure A1.4, once the running wheel was introduced (day 16), rats immediately began running with their activity on the first full day reaching an average of 3293.67 wheel rotations ($SEM = 574.55$). Running wheel activity remained stable across the first 4 days of the restricted feeding-active phase ($F(3, 15) = 1.01, p = .418, \eta_p^2 = .17$). Visual inspection of the data presented in Figure 3.4, however, suggests that running wheel activity began to increase by the 4th day of this phase. Indeed, by the 4th day, rats ran an average of 4370.00 wheel rotations ($SEM = 1112.55$).

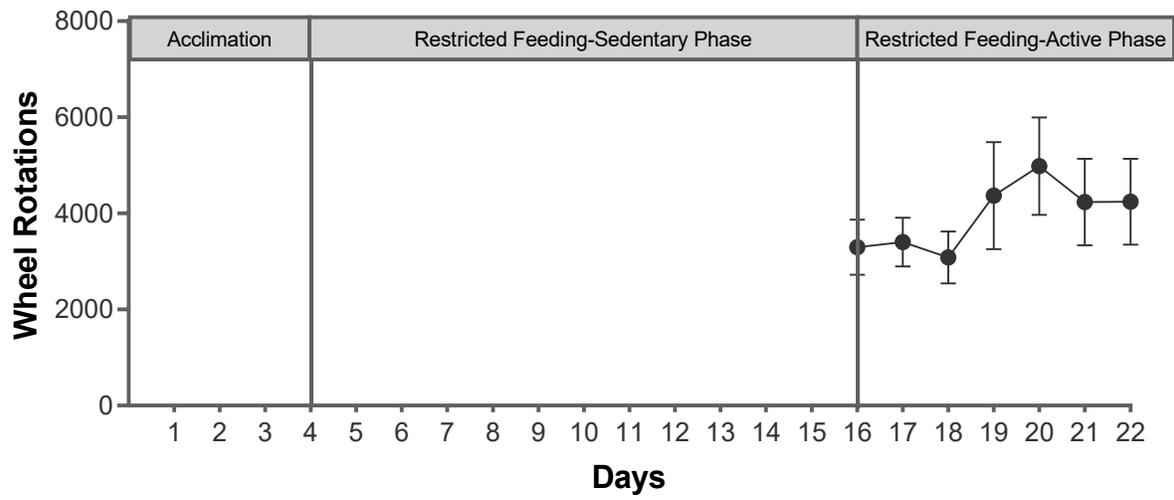


Figure A1.4. Daily running wheel activity under a restricted-feeding schedule.

Discussion

To the best of our knowledge, the present experiment is the first study examining ABA in Long-Evans male rats and investigating the effect of pre-exposure to a 1 h/day feeding schedule on the development of ABA. We expected that pre-exposing rats to the feeding schedule would allow them to adapt and stabilize their body weight. During the restricted feeding-active phase, however, we expected that food intake would eventually stabilize at pre-wheel levels but that rats would continue to lose weight and reach removal criterion, despite pre-exposure to the feeding schedule.

Rats were unable to stabilize their weight during the pre-exposure period

An important finding from this experiment was that sedentary Long-Evans rats were unable to adapt, and thus stabilize their weight, when feeding was restricted to 1 h/day. While food intake gradually increased across the restricted feeding-sedentary phase, all rats continued to lose weight, reaching a group average of 76% of their initial body weight by the 11th day of pre-exposure, at which point the phase was ended. This finding contrasts with previous findings in which rats were able to stabilize their weight over time when allowed to adapt to a feeding schedule of 1.5 h/day (Dwyer & Boakes, 1997; Lett et al., 2001). Dwyer and Boakes (1997) tested the effect of pre-exposure to a 1.5 h/day feeding schedule with male albino rats randomly divided into a preadapted and a nonadapted group. Similarly to our results, the authors found that the beginning of the pre-exposure phase resulted in an initial drop in food intake. However, unlike our results, this initial decrease was followed by a gradual increase in food intake which was sufficient to allow weight gain after 14 days. Our results also contrast with those of Lett et al. (2001) who indicated that male Sprague Dawley rats pre-exposed to a feeding schedule of 1.5 h/day were able to stabilize their weight by the 13th day of pre-exposure. Our finding also appears to be inconsistent with Routtenberg and Kuznesof (1967)'s report that albino male rats who were sedentary were able to maintain their body weight on a 1 h/day feeding schedule. While the authors did not directly investigate the effect of pre-exposure to the feeding schedule on the development of ABA, they exposed rats to a 1 h/day feeding schedule and compared the food intake of active rats to control sedentary rats. They found that 4 out of 5 active rats starved while only 1 out of 5 sedentary control rats starved, suggesting that the 4 remaining control rats were able to maintain their body weight (Routtenberg & Kuznesof, 1967). There are various

explanations as to why the rats in the present study were unable to stabilize their body weight during pre-exposure to the feeding schedule including the severity of the feeding schedule, age of animals, and strain effects.

Feeding schedule. One possible explanation is that access to food for 1 h/day is simply too little time to enable male rats to consume enough calories to maintain their body weight even when sedentary. While the 1 h/day feeding schedule is now common practice in ABA experiments in female rats, there are, to the best of our knowledge, only two previous studies that examined ABA in male rats using a 1 h/day feeding schedule. As described above, Routtenberg and Kuznesof (1967) suggested that 4 out of 5 sedentary control rats on a 1 h/day feeding schedule were able to maintain their body weight, thereby avoiding starvation. It should be noted that the pre-exposure phase lasted 11 days in our experiment while the sedentary control rats in Routtenberg and Kuznesof (1967)'s study were exposed to the feeding schedule for 7 days only. In their experiment, sedentary control rats ate approximately 10 g of food on the 7th day which is comparable to the 10.17 g our rats were eating on the 7th day of preexposure.

Ratnovsky and Neuman (2011) carried out the only experiment that was designed, in part, to examine the effect of pre-exposure to a 1 h/day feeding schedule on ABA in male rats (Harlan Sprague Dawley). In their study, rats were kept in the pre-exposure phase until they reached weight stability criterion. While the authors do not report how many days were required for rats to reach stability criterion, they report that, unlike our rats, all rats were eventually able to stabilize their weight under this feeding schedule. There is, however, an important discrepancy between our pre-exposure procedures. In our experiment, pre-exposure was terminated on the 11th day when rats, on average, weighed 75% of their initial body weight and it was assumed that they would not be able to stabilize even if given more days. In the Ratnovsky and Neuman (2011) experiment, when rats weighed $\geq 75\%$ of their initial body weight for two consecutive days, they were given *ad libitum* access to food until their weight rose above 75% of their initial body weight for two days and then were placed back on the 1 h/day feeding schedule until they reached weight stability criteria. Thus, their procedure suggests that their rats may not have been able to maintain their body weight had they not been given the opportunity to regain their weight through *ad libitum* feeding. Based on our findings and the details highlighted above from the Routtenberg and Kuznesof (1967) and Ratnovsky and Neuman (2011) studies, it would appear

that male rats are unable to maintain their body weight and avoid starvation (when defined as $\leq 75\%$ of initial body weight) when kept on a continuous 1 h/day feeding schedule. More gradual or flexible pre-exposure procedures (e.g., Ratnovsky & Neuman, 2011) appear to be necessary to promote weight stability under such a severe food restriction. Such caution may not be necessary with the less severe food restriction of 1.5 h/day (e.g., Dwyer & Boakes, 1997; Lett et al., 2001).

Age of animals. The age of the subjects used is another important factor that may help explain discrepancies across studies. The rats used in the present experiment ranged between approximately postnatal days (PD) 63 and 98, corresponding to early to mid-adulthood. Routtenberg and Kuznesof (1967) as well as Dwyer and Boakes (1997) used a particularly wide age range of approximately PD49 to PD105 which encompasses adolescence, early adulthood, and mid-adulthood. Lett et al. (2001) used rats that were adolescents and early adults (PD57-91). Ratnovsky and Neuman (2011)'s subjects consisted of particularly younger rats in adolescence and early adulthood (PD28-70). Our study is thus the only experiment that did not include adolescent rats. ABA has been shown to develop more rapidly in adolescent rats compared to adult rats (Frintrop et al., 2018), which may lead one to assume that adult rats would more easily adapt during pre-exposure to a food restriction schedule. The increased vulnerability of adolescent rats to ABA, however, is not the result of lower food intake, but rather of more intense running wheel activity which results in rapid weight loss (Frintrop et al., 2018). When sedentary, it is possible that adult rats, naturally weighing more than adolescent rats, have more difficulty consuming the number of calories needed to prevent starvation in a 1 h time window. As mentioned above, on the 7th day of pre-exposure, our adult rats consumed an average of 10.17 g a day which is comparable to the daily amount eaten by the rats in the Routtenberg and Kuznesof (1967) study which included adolescent rats. It is possible that 10 g of food per day was enough to prevent starvation in younger (i.e., lighter) rats, but not in adult (i.e., heavier) rats.

Strain differences. Rat strain may also influence how a rat adapts to a feeding schedule. The early ABA experiments, and those examining the impact of pre-exposure to the feeding schedule, have been conducted in albino rats (Dwyer & Boakes, 1997; Routtenberg & Kuznesof, 1967) or, more specifically, Sprague Dawley rats (Lett et al., 2001; Ratnovsky & Neuman, 2011). To the best of our knowledge, the present study is the only experiment investigating ABA

in Long-Evans male rats. While strain comparisons of ABA have been conducted in mice, this has not been the case in rats. Based on the present study and many studies from our laboratory using adult Long-Evans males, we know that these rats typically eat approximately 30 g/day of regular chow when they have *ad libitum* access to food and are sedentary. From the data available in the Dwyer and Boakes (1997) publication, it appears that albino rats who are sedentary and have *ad libitum* access to food ate between 25-28 g/day. It is thus possible that Long-Evans rats require more calories to maintain their body weight compared to albino strains, making it more challenging for them to stabilize their body weight under a strict feeding schedule. In one experiment aimed at investigating vendor differences in alcohol consumption in Long-Evans rats, researchers found that rats purchased from Charles Rivers gained more weight (and thus presumably consumed more calories) than rats of the same strain purchased from Harlan (Sparks, Sciascia, Ayorech, & Chaudhri, 2014). If such important differences can exist between same-strain rats obtained from different vendors, one can assume that there may be important strain differences in food intake which may help explain why some rats can survive food restriction while others cannot. Strain comparison studies in ABA should thus be performed in rats.

Trending wheel-induced feeding suppression

We observed a trending, but not statistically significant, decrease in food intake for 4 days after the wheel was introduced. Such wheel-induced feeding suppressions in food restricted rats have previously been described by other researchers. For instance, when Routtenberg (1968) examined the effect of pre-exposure to a 1h/day feeding schedule on the development of ABA, he reported that wheel introduction resulted in reduced food intake in the preadapted rats both compared to their pre-wheel feeding levels and compared to feeding levels of the control rats (not pre-exposed to the feeding schedule). Dwyer and Boakes (1997) reported that wheel access resulted in a reduction in food intake in rats on a 1.5 h/day feeding schedule. They reported, however, that this slight depression in food intake quickly recovered (after approximately 4 days) and went on to exceed pre-running levels. Unfortunately, given that our observed decrease in food intake did not reach statistical significance, it is difficult to determine whether food intake returned to pre-running levels. Consistent with reports of wheel-induced feeding suppression, Lett et al. (2001) also found that rats pre-exposed to the 1.5 h/day feeding schedule showed

suppressed feeding when the wheel was introduced compared to pre-exposed rats that were not given access to the wheel. Since we did not have a pre-exposed and sedentary control group, as in Lett et al. (2001), we were unable to make such a between-group comparison. However, the trending decrease in food intake within subjects over time may be in line with findings reported by Lett et al. (2001).

Introduction of the running wheel did not accelerate weight loss

We hypothesized that, despite pre-exposure to the feeding schedule, wheel introduction would result in high levels of activity which, in tandem with reduced food intake, would result in accelerated weight loss. This hypothesis was only partially supported. As described above, we observed higher levels of running wheel activity than those previously reported in similar experiments (Dwyer & Boakes, 1997; Lett et al., 2001). This hyperactivity paired with restricted food intake was accompanied by continuous weight loss that would have been life-threatening had the experiment not been terminated. Contrary to our hypothesis, however, we did not observe accelerated weight loss. Instead, rats continued to lose weight at the same rate as before the wheel was introduced. While we can say that ABA developed as rats were hyperactive and did not consume enough food to compensate and survive, we cannot rule out the possibility that subjects could have survived had they had sufficient time to adapt to the feeding schedule prior to wheel introduction (i.e., adaptation theory). In addition, it is possible that the hyperactivity observed in our rats compared to those from other experiments was driven by the fact that the rats in our experiment were already at a critically low body weight by the end of the pre-exposure phase because they had been unable to adapt to the feeding schedule. Because no control group was used in this experiment, we cannot rule out the possibility that body weight at the time of wheel introduction effected levels of running wheel activity and subsequent weight loss. We can also not rule out the possibility that pre-exposure to the feeding schedule, despite it not resulting in adaptation in our hands, may have slowed the ABA effect. In order to rule out these possibilities, future studies should include a control condition in which rats are not pre-exposed to the feeding schedule before starting the ABA phase.

Individual variability in ABA

Despite our small sample size which was undoubtedly a limitation to the present experiment, we were able to observe individual variances which warrant more attention. We

found that one rat ate particularly small amounts of food and showed high levels of running wheel activity which resulted in premature removal from the experiment after only 4 days of the restricted feeding-active phase while another rat appeared to compensate for its running wheel activity by eating particularly larger amounts of food allowing it to stabilize its body weight during the restricted feeding-active phase. With such a small sample size, it is impossible to determine whether such animals represent statistical outliers or are indicative of two distinct populations (resilient vs. susceptible rats).

Conclusion

In summary, we found that Long-Evans male rats in the present experiment were unable to stabilize their body weight when sedentary whilst maintained on a 1 h/day feeding schedule suggesting that this schedule may not be ideal for this rat strain. We found that rats showed increasing levels of wheel activity once the wheel was introduced and that, despite being pre-exposed to the feeding schedule, rats did not increase their food intake, resulting in an inability to compensate for their increased energy expenditure. We did observe a trending wheel-induced feeding suppression and individual variability in ABA susceptibility which merit further investigation.

APPENDIX 2: THE EFFECT OF PRE-EXPOSURE TO THE RUNNING WHEEL ON THE DEVELOPMENT OF ABA

In our preliminary study presented in Appendix 1, rats were pre-exposed to the restricted feeding schedule before the running wheel was introduced. In the present experiment, we examined the effect of pre-exposure to the running wheel prior to food restriction on levels of activity, food intake, and body weight.

Hyperactivity, in the context of severe food restriction, is one of the key components of the ABA model. In typical ABA experiments, the onset of a restricted feeding schedule results in significant increases in running wheel activity which interferes with food intake resulting in rapid weight loss. As expected, our preliminary findings (presented in Appendix 1) indicated heightened levels of running wheel activity despite being food restricted. We were, however, unable to determine if food restriction resulted in an increase in running wheel activity because the restricted feeding schedule was initiated prior to the introduction of the running wheel. Thus, in the present experiment, we sought to examine whether running wheel activity would increase upon food restriction by pre-exposing rats to the running wheel and introducing food restriction once wheel activity had stabilized.

Another goal of the present experiment was to examine how many days Long-Evans male rats could survive in the ABA procedure. In the preliminary experiment presented in Appendix 1, the ABA phase was terminated after 7 days as rats had, on average, lost more than 25% of their initial body weight. It is likely, however, that the ABA phase was particularly short because rats had already begun to lose weight during the food restriction pre-exposure phase prior to the onset of the ABA phase. In the present experiment in which rats were not food restricted until the start of the ABA phase, we expected to see a longer survival period.

Pre-exposure to the running wheel in the present experiment would also enable us to assess the effect of wheel introduction on food intake under stated conditions. We previously observed a trending decrease in food intake lasting 4 days following wheel introduction. Wheel-induced feeding suppression has previously been reported in food restricted rats (e.g., Dwyer & Boakes, 1997; Routtenberg, 1968). Wheel introduction has also been reported to induce a temporary feeding suppression in sated rats. For instance, Afonso and Eikelboom (2003) found that wheel access was associated with a 25% decrease in food intake in Sprague Dawley males

who were sated relative to food intake of sated and sedentary rats. They reported that this effect lasted at least 8 days but was followed by a 13% increase in food intake relative to the sedentary control rats (Afonso & Eikelboom, 2003). Their findings were consistent with earlier work demonstrating that wheel access temporarily suppresses feeding and chronically reduces body weight (Goodrick et al., 1983; Looy & Eikelboom, 1989). We were therefore interested in examining whether a temporary wheel-induced feeding suppression would be observed, and for how many days, in Long-Evans male rats.

Finally, the present experiment was designed to examine what would happen to food intake, running wheel activity, and body weight when rats are allowed to recover (i.e., given *ad libitum* food) following the ABA procedure. To the best of our knowledge, Ratnovsky and Neuman (2011) are the only researchers to have examined recovery following ABA in rats. They found that Harlan Sprague Dawley males given free access to food reduced their running wheel activity, experienced hyperphagia (relative to baseline) and regained their body weight (Ratnovsky & Neuman, 2011). In the present experiment, we examined whether similar results would be obtained in Long-Evans male rats.

To explore these questions, a group of Long-Evans male rats was given pre-exposure to the running wheel followed by an ABA phase (food restriction and wheel access) and a recovery period in which they had unlimited access to food. During the wheel pre-exposure phase, we hypothesized that introduction of the wheel would result in a temporary decrease in food intake and that running wheel activity would eventually stabilize. During the ABA phase, we hypothesized that the beginning of food restriction would result in an increase in running wheel activity and accelerated weight loss. Finally, during the recovery phase, we hypothesized that rats would reduce their running activity, increase their food intake, and recover their body weight.

Method

Subjects

Male Long-Evans rats ($n = 6$; 325-350 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec) and housed in a colony room on a 12:12 hr reverse light/dark cycle. Upon arrival, rats were initially pair-housed in plastic shoebox cages and were

then separated into individual shoebox cages (experimental day 1). On day 4, rats were transferred to the laboratory where they were permanently housed in running wheel cages inside sound-attenuating boxes until the end of the experiment. Body weight (g), food intake (g), and water intake (g) was monitored daily at ZT 11-12. According to the survival guidelines offered by Routtenberg and Kuznesof (1967), rats were removed from the experiment once they reached survival criteria of daily food intake below 1 g.

Apparatus

Running Wheel Cages. See “General Methodology” section.

Procedure

The detailed timeline is depicted in Figure A2.1.

Acclimation phase (days 1-4). Rats were individually housed in plastic shoebox cages for 4 days in the Animal Care Facility, allowing them time to acclimate to the new environment. This period also allowed for baseline daily measures of body weight, food intake, and water intake.

Running wheel-sated phase (days 5-15). During this phase lasting 11 days, rats had continuous access to the running wheel and simultaneous *ad libitum* access to food and water. The purpose of this phase was to allow the rats to habituate to the running wheels prior to initiating the restricted feeding schedule. Furthermore, this phase provided a baseline measure of running wheel activity.

Running wheel-restricted feeding phase (days 16-23). On the first day of this 7-day phase, food was removed at ZT 13. On the following days, rats had access to food for 1 hr/day between ZT 12-13. Importantly, rats continued to have continuous access to the running wheel throughout this phase.

Recovery phase (days 24-29). Following 7 days of restricted feeding, rats in this condition were given 6 days of *ad libitum* access to food while continuing to have access to the running wheel. The purpose of this phase was to examine what would happen to rats’ food intake, body weight, and running wheel activity if given the opportunity to recover.

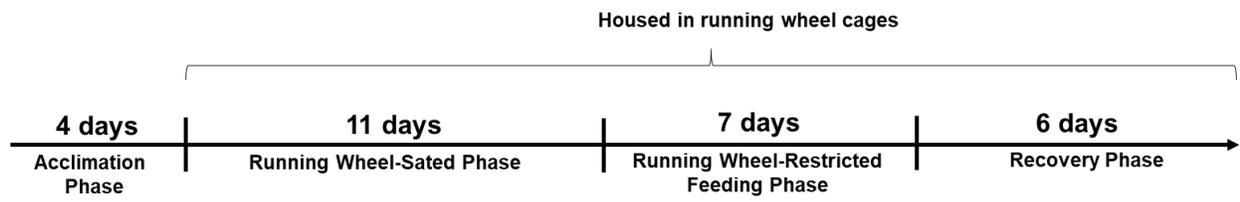


Figure A2.1. Timeline of supplemental experiment 2.

Statistical Analysis

Food intake. To assess for changes in food intake from the acclimation phase to the running wheel-sated phase, a within-subjects *t*-test was used comparing food intake averaged across the 5 days of the acclimation phase with food intake averaged across the first 5 days of the running wheel-sated phase. Another *t*-test was then used to compare food intake on the last day of the acclimation phase versus the last day of the running wheel-sated phase. To examine food intake during the running wheel-restricted feeding phase, a repeated-measures ANOVA was used across the 8 days of this phase. A repeated-measures ANOVA was also used to examine food intake across the 6 days of recovery. Finally, a within-subjects *t*-test was used to compare average food intake across the 5 days of acclimation versus the 5 first days of recovery.

Running wheel activity. A repeated-measures ANOVA was used to characterize running wheel activity across the 11 days of the running wheel-sated phase. To determine whether activity stabilised by the end of this phase, an ANOVA was used across the last 4 days of the running wheel-sated phase. A *t*-test was used to compare the averaged wheel rotations during the last 4 days of the running wheel-sated phase to the first 4 days of the running wheel-restricted feeding phase. Another *t*-test compared the averaged wheel rotations across the last 5 days of the running wheel-restricted feeding phase to the first 5 days of recovery. Finally, a repeated-measures ANOVA was used to characterize the change in wheel running across the first 5 days of recovery.

Body weight. A repeated-measures ANOVA was used to characterize body weight across the first 4 days of the acclimation phase and a *t*-test compared body weight on the last day of acclimation to the first day of the running wheel-sated phase. To examine body weight during the running wheel-sated phase, a repeated-measures ANOVA was used across the 11 days of this phase and a *t*-test compared body weight on the last day of this phase to the first day of the running wheel-restricted feeding phase. A repeated-measures ANOVA was used across the 7 days of the running wheel-restricted feeding phase and a *t*-test compared body weight on the last day of the running wheel-restricted feeding phase to the first day of the recovery phase. Finally, a repeated-measures ANOVA was used to characterize body weight across the 6 days of recovery.

Results

Data Integrity

As no outliers were identified across all variables of interest, all 6 rats were included in the analyses. One rat was removed from the experiment on day 21 (6th day of the running wheel-restricted feeding phase) due to severe starvation symptoms. As such, any analyses of food intake, body weight, and activity during the recovery phase included 5 rats out of 6.

Food intake

As can be seen in Figure A2.2A, the introduction of a running wheel under *ad libitum* feeding conditions resulted in a decrease in food intake. Indeed, food intake during the running wheel-sated phase appeared to be lower than food intake during the acclimation phase, though this decrease did not reach statistical significance ($t(5) = 1.95, p = .054, d = 0.80$, Figure A2.2B). This trending wheel-induced decrease in food intake lasted approximately 6 days with rats eating an average of 25.33 g ($SEM = 2.50$) on the 6th day of the running wheel-sated phase compared to 32.33 g ($SEM = 2.96$) on the day before the wheel was introduced. There was no difference in food intake between the last day of the running wheel-sated phase and the last day of the acclimation phase before the wheel was introduced ($t(5) = 0.83, p = .445, d = 0.34$; Figure A2.2C).

Not surprisingly, food restriction resulted in a severe decrease in food intake from 29.17 g ($SEM = 2.85$) on the final day of the running wheel-sated phase to a mean of 7.83 g ($SEM = 1.51$) on the first day of the running wheel-restricted feeding phase. As can be seen in Figure 3.6A, there was a gradual and statistically significant increase in food intake across restricted-feeding days ($F(2, 28) = 7.44, p < .001, \eta_p^2 = .65$).

On the first day of the recovery phase, reintroduction of *ad libitum* food resulted in a sharp increase in food intake reaching a mean of 34.80 g ($SEM = 2.37$) on the first day of recovery. Daily food intake appeared to increase gradually across recovery days, peaking at a mean of 46.60 g ($SEM = 3.91$) by the last day of recovery, though this gradual increase was not statistically significant ($F(5, 20) = 2.34, p = .079, \eta_p^2 = .37$; Figure A2.2A). Food intake during the recovery phase was higher than baseline food intake during the acclimation phase ($t(4) = -3.35, p = .029, d = 1.50$; Figure A2.2D).

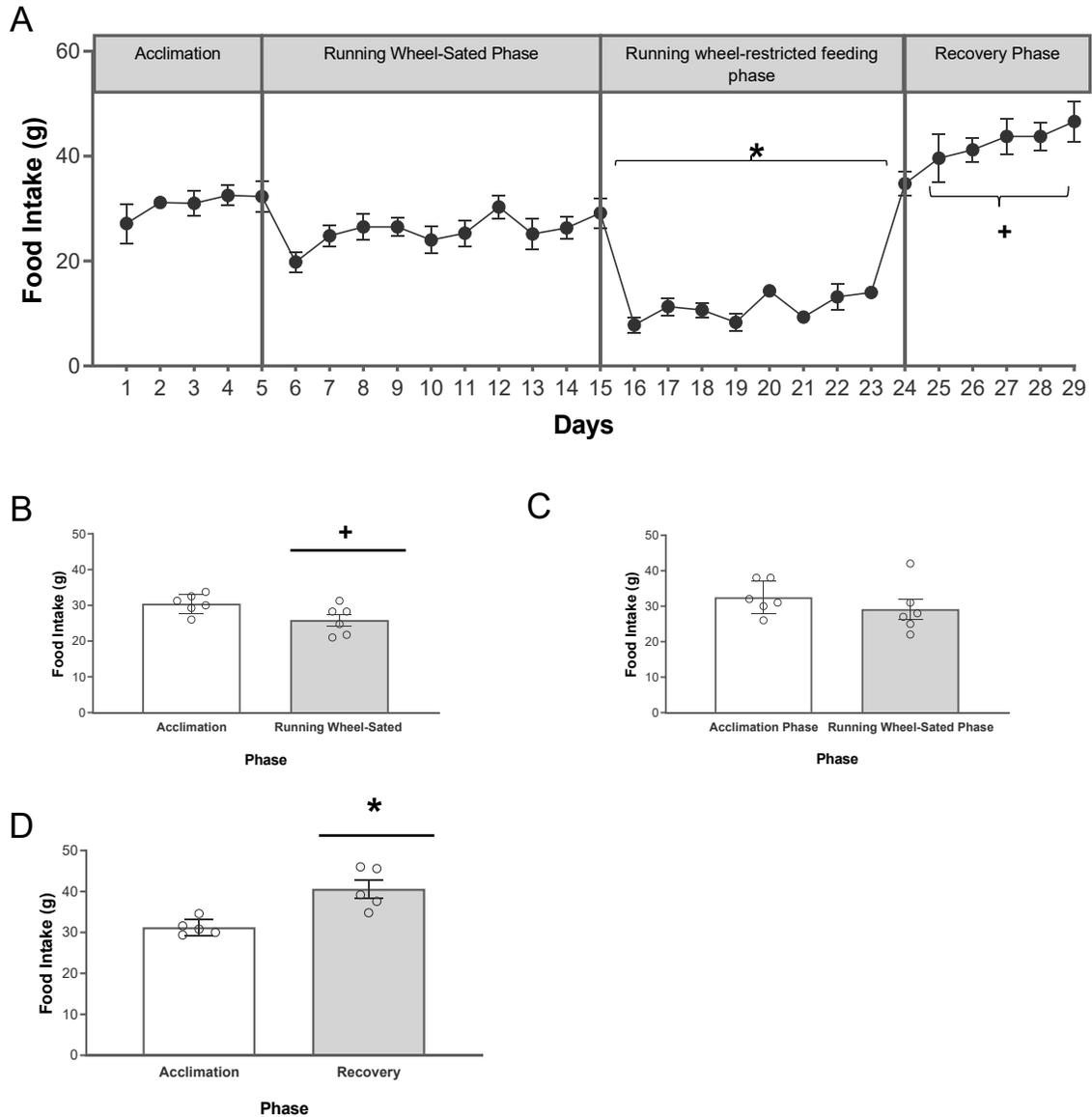


Figure A2.2. The effect of food restriction on daily food intake in rats with access to a running wheel. (A) Daily food intake across experimental days, $*p < .001$; $+p = .079$. (B) Average daily food intake across the 4 days of the acclimation phase compared to the first 4 days of the running wheel-sated phase, $+p = .054$. (C) Average daily food intake on the last day of the acclimation phase compared to the last day of the running wheel-sated phase. (D) Average daily food intake across the 5 days of acclimation compared to the 5 days of recovery, $*p = .029$

Running wheel activity

As can be seen in Figure A2.3A, rats began to use the running wheel on the first day it was introduced under sated conditions and their running wheel activity increased in a statistically significant way across the 11 days of the running wheel-sated phase ($F(10, 50) = 9.65, p < .001, \eta_p^2 = .66$). Rats' daily wheel rotations stabilized at a daily mean of 3877.83 wheel rotations ($SEM = 609.91$) across the final 4 days of the running wheel-sated phase ($F(3, 15) = 1.86, p = .180, \eta_p^2 = .27$).

The beginning of the running wheel-restricted feeding phase resulted in an increase in running wheel activity that can be seen in Figure A2.3A. Indeed, the wheel rotations averaged across the first 4 days of the running wheel-restricted feeding phase was higher than the wheel rotations averaged across the last 4 days of the running wheel-sated phase ($t(5) = -2.60, p = .048, d = 1.06$; Figure A2.3B). Running wheel activity reached a peak of 8075.17 average daily rotations ($SEM = 1815.91$) on the fourth day of the restriction phase.

Reintroduction of *ad libitum* food during the recovery phase resulted in a sharp decrease in running wheel activity that can be seen in Figure A2.3A. Indeed, running wheel activity was significantly lower during the 5 days of recovery compared to the average of the last 5 days of the running wheel-restricted feeding phase ($t(4) = 12.08, p < .001, d = 5.40$; Figure A2.3C). While rats' daily running wheel activity reached an all-time low on the first day of recovery ($M = 620.60, SEM = 117.22$), their daily running wheel activity increased across the 5 recovery days ($F(4, 16) = 9.23, p < .001, \eta_p^2 = .70$).

Body weight

Rats showed a gradual and statistically significant increase in body weight during the acclimation phase ($F(3, 15) = 25.69, p < .001, \eta_p^2 = .84$; Figure A2.4A). The introduction of the running wheel resulted in a statistically significant drop in body weight from the last day of the acclimation phase to the first day of the running wheel-sated phase ($t(5) = 2.95, p = .016, d = 1.21$; Figure A2.4B). Following this initial weight loss, body weight remained stable across the 11 days of the running wheel-sated phase ($F(10, 50) = 0.73, p = .689, \eta_p^2 = 0.13$; Figure A2.4A).

Not surprisingly, food restriction resulted in statistically significant weight loss when comparing body weight on the last day of the running wheel-sated phase compared to the first

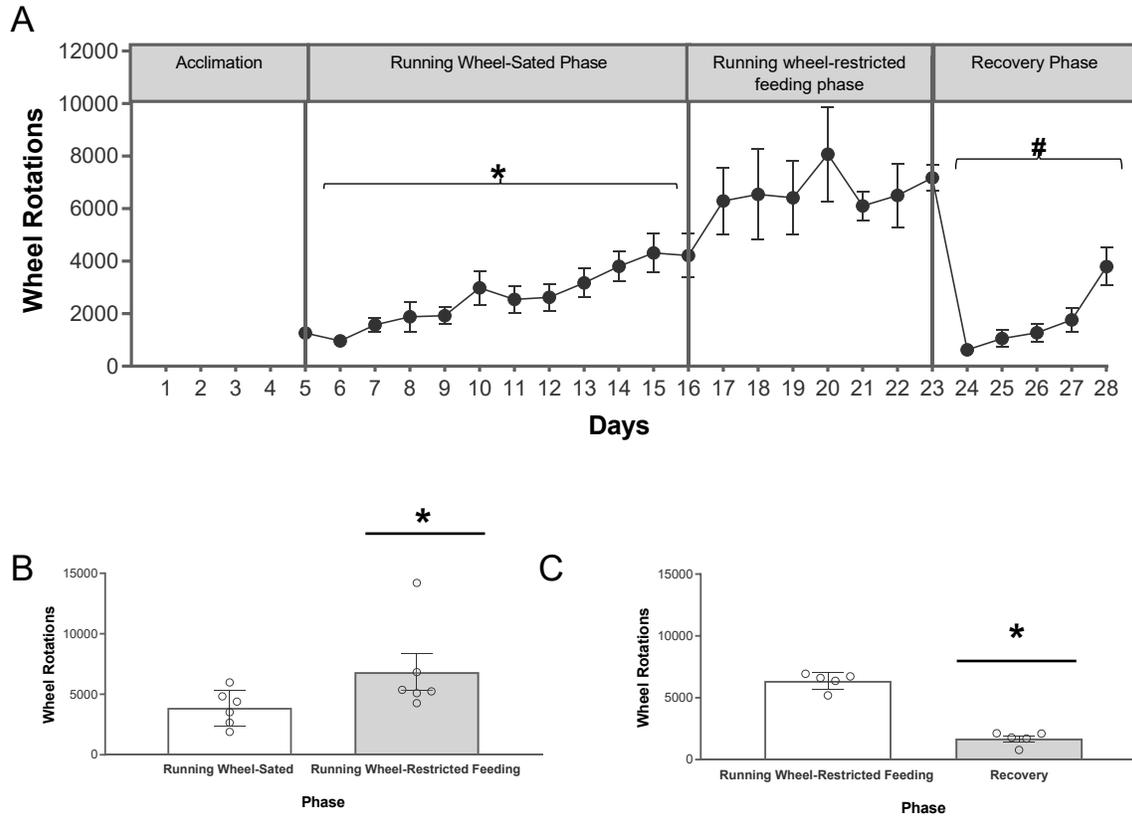


Figure A2.3. The effect of food access on running wheel activity. (A) Running wheel activity across experimental days, $*p < .001$, main effect of *days* during the running wheel-sated phase $\#p < .001$; main effect of *days* during the recovery phase. (B) Average running wheel activity across the last 4 days of the running wheel-sated phase compared to the first 4 days of the running wheel-restricted feeding phase, $*p = .048$. (C) Average running wheel activity across the last 5 days of the running wheel-restricted feeding phase compared to the 5 days of recovery, $*p < .001$.

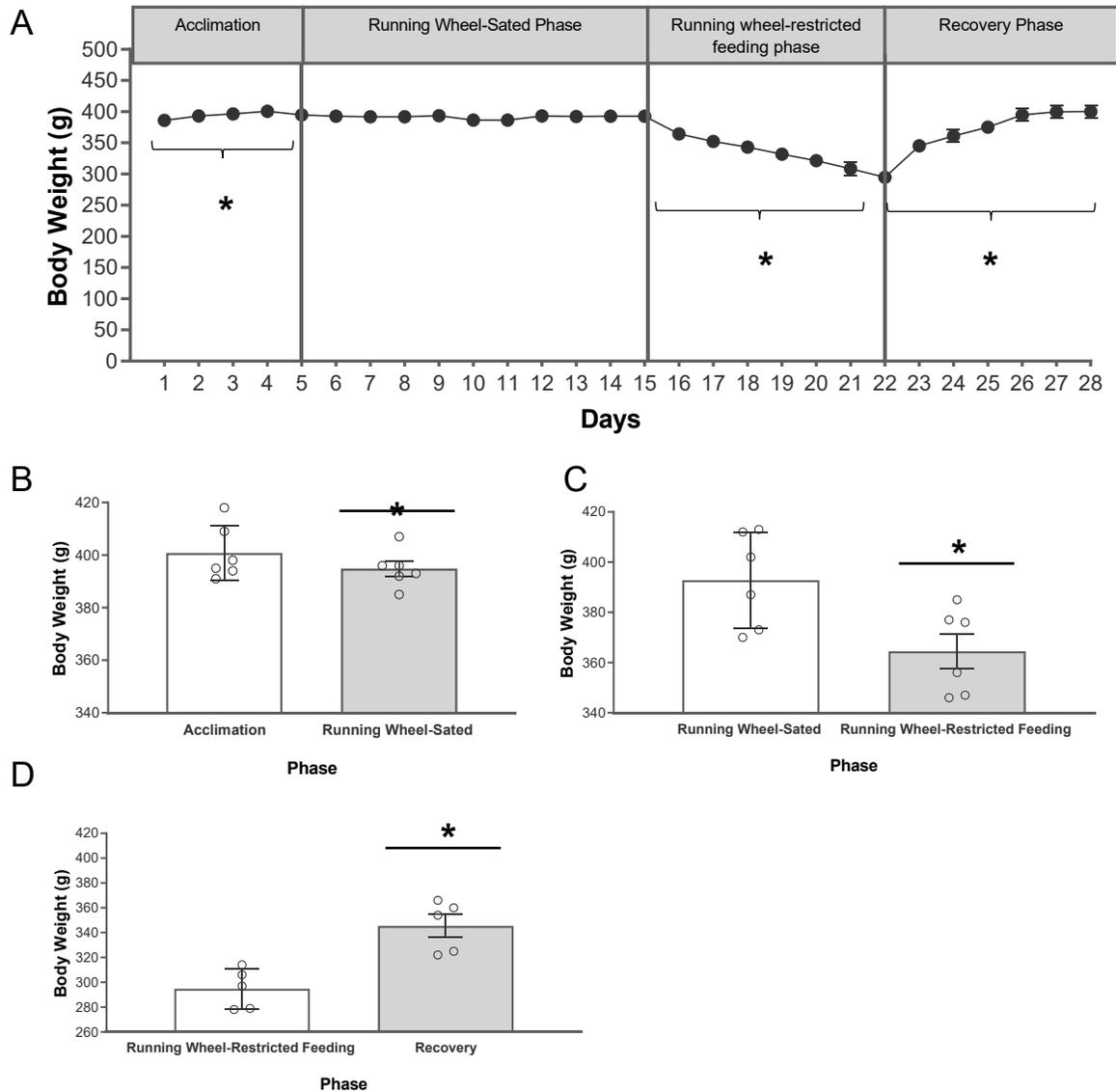


Figure A2.4. The effect of food restriction and running wheel activity on body weight. (A) Body weight across experimental days, $*p < .001$, main effect of days during the acclimation, running wheel-restricted feeding phase, and recovery phase. (B) Average body weight on the last day of acclimation compared to the first day of the running wheel-sated phase, $*p = .016$. (C) Average body weight on the last day of the running wheel-sated phase compared to the first day of the running wheel-restricted feeding phase, $*p < .001$. (D) Average body weight on the last day of the running wheel-restricted feeding compared to the first day of recovery, $*p < .001$.

day of the running wheel-restricted feeding phase ($t(5) = 14.98, p < .001, d = 6.12$; Figure 3.8C). Rats continued to lose weight in a statistically significant way across the 7 days of the running wheel-restricted feeding phase ($F(6, 24) = 166.17, p < .001, \eta_p^2 = 0.98$; Figure A2.4A).

Reintroduction of *ad libitum* food during recovery resulted in immediate and statistically significant weight gain when comparing body weight on the last day of the running wheel-restricted feeding phase to that of the first day of the recovery phase ($t(4) = 20.73, p < .001, d = 9.27$; Figure 3.8D). Body weight continued to increase across the 6 days of recovery ($F(5, 20) = 51.52, p < .001, \eta_p^2 = 0.93$; Figure A2.4A).

Discussion

The main purpose of the present experiment was to examine whether Long-Evans male rats pre-exposed to the running wheel would develop and recover from ABA. Different sets of hypotheses were outlined for the various experimental phases.

Wheel habituation phase

It was hypothesized that the introduction of the running wheel under stated conditions would result in a temporary reduction in food intake. Indeed, we observed a wheel-induced feeding suppression lasting approximately 6 days. While this suppression did not reach statistical significance, the large effect size suggests that increasing our sample size, thereby increasing our statistical power, may have resulted in a statistically significant effect. We found that the introduction of the running wheel, accompanied by the trending reduction in food intake, resulted in initial weight loss suggesting that rats were not consuming enough calories to compensate for their increased energy expenditure.

Our findings were partially consistent with those of Afonso and Eikelboom (2003) who reported a 25% decrease in food intake (relative to a sedentary control group) upon wheel introduction lasting 8 days. Although they reported a longer wheel-induced feeding suppression, they also reported that this effect was eventually reversed and that rats that had access to the wheel eventually ate 13% more than the sedentary control rats. Although a sedentary control group was not included in the present experiment, within-subjects comparisons suggested that

food intake in our rats eventually returned to baseline levels but did not exceed these levels. In other words, the rats in the present experiment were not consuming enough calories to promote weight gain. It should be noted that the wheel pre-exposure phase used by Afonso and Eikelboom (2003) lasted 32 days, compared to 11 days in the present experiment, and that the 13% increase in food intake was observed during the last days of this phase. This would suggest that the rats in the present experiment may have eventually increased their intake had the phase lasted longer. Ratnovsky and Neuman (2011) also observed a temporary wheel-induced feeding suppression. In their case, the reduction lasted 3 days before returning to baseline feeding levels and was accompanied by an initial stabilization in body weight before returning to gradual weight gain. Thus, it appears that the trending wheel-induced feeding suppression observed in the present experiment may suggest that Long-Evans males not only show a wheel-induced feeding suppression but are unable to increase their feeding over time to compensate, thus interfering with subsequent weight gain.

We had also hypothesized that rats would show increasing levels of running wheel activity which would eventually stabilize. Consistent with this hypothesis, we found that running wheel activity increased across the pre-exposure phase, reaching stability by the 11th day of this phase. Rats took to the running wheel immediately upon its introduction, recording an average of 1264 rotations on the first day and reaching 3878 daily rotations by the end of the pre-exposure phase. These numbers were consistent with findings by Afonso and Eikelboom (2003) who reported an average of 1031 wheel rotations on the first day and 3053 daily rotations on day 8. Ratnovsky and Neuman (2011), however, reported activity levels below 1000 daily rotations during the wheel pre-exposure phase. Interestingly, during this same phase, their rats were eating in the 20-30 g/day range which was comparable to the food intake of our subjects who were significantly more active. This may help explain why the rats in the present experiment only managed to maintain their body weight after an initial weight loss while those in Ratnovsky and Neuman's (2011) experiment were able to gain weight.

ABA phase

Giving rats pre-exposure to the running wheel prior to beginning food restriction enabled us to test the hypothesis that food restriction would result in hyperactivity, a crucial element of the ABA model. Indeed, we found that the onset of food restriction resulted in a statistically

significant increase in running wheel activity compared to pre-food restriction levels. While we did observe an increase in food intake across the running wheel-restricted feeding phase, this increase was insufficient to compensate for the increased energy expenditure, resulting in significant weight loss that continued across this phase. Consistent with our findings, Routtenberg (1968) had found that rats continued to lose weight throughout ABA despite increasing their food intake across days. Routtenberg (1968)'s design included a sedentary control condition in which food restricted rats did not have access to the running wheel. This comparison allowed them to demonstrate that active rats ate significantly less than sedentary rats during ABA and that weight loss was accelerated in the active rats (i.e., anorectic effect). While our findings appear to be in line with those of Routtenberg (1968), a sedentary control group would be necessary to confirm the presence of an anorectic effect.

In our preliminary study (presented in Appendix 1), we had found that rats reached starvation criterion after 7 days of the ABA phase. Those rats, however, had already lost weight during the pre-exposure to the feeding schedule phase prior to the beginning of ABA. Because rats in the present experiment were sated until the onset of ABA, we had hypothesized that they would survive ABA for more than 7 days. This hypothesis was not supported as rats reached starvation criterion by the 7th day of ABA, with one rat even reaching this criterion by day 6. Given that Long-Evans male rats appear to show a strong ABA effect, it would be interesting to investigate whether increasing the feeding window to 90 minutes (instead of 60 minutes used here) would extend the ABA phase while enabling the ABA effect.

It should be noted that on the 7th day of the running wheel-restricted feeding phase, when rats, on average, weighed 75% of their initial body weight, they ate an average of 14 g. Thus, it is highly likely that rats in the original ABA studies (Routtenberg & Kuznesof, 1967; Routtenberg, 1968) had lost significantly more weight when they reached the starvation criterion of ≤ 1 g of food/day. Knowing that rats have been shown to develop stomach ulcers after losing $\geq 30\%$ of their initial weight loss (Doerries et al., 1991), the starvation criterion of ≤ 1 g of food/day is too severe and would result in the inclusion of rats whose food intake and activity is confounded by the presence of stomach ulcers. This observation lends support to the contemporary practice of using a 25% weight loss as the cut-off or starvation criterion in ABA studies.

Recovery Phase

It was hypothesized that a recovery phase would result in a reduction in running wheel activity, hyperphagia, and weight gain. This hypothesis was partially supported. We found that the reintroduction of food resulted in an increase in food intake with a trending increase across days reaching amounts that surpassed baseline food intake. In addition to this increased food intake, we observed an immediate decrease in running wheel activity. In fact, the lowest level of daily running across the entire experiment was recorded on the first day of recovery. Running activity did increase across recovery days but remained lower than the levels reached during the running wheel-sated phase. The reduction in running activity and accompanying hyperphagia resulted in weight gain whereby rats had fully recovered their baseline body weight by the end of recovery. These results are consistent with those of Ratnovsky and Neuman (2011) and illustrate that access to food allows for almost immediate weight recovery despite continued access to a running wheel. Ratnovsky and Neuman (2011) make the interesting suggestion that this recovery phenomenon may be relevant to treatment programs in the human clinical population that often do not allow patients to exercise to promote faster weight gain. The inability to exercise during treatment, albeit an effective way to promote weight gain, often makes it difficult for patients to fully comply with treatment resulting in increased drop out rates (Bandini et al., 2006; Zeeck, Hartmann, Buchholz, & Herzog, 2005). Ratnovsky and Neuman (2011) make the argument that treatment compliance may increase if patients with AN receiving treatment and consuming the appropriate amount of daily calories are allowed to engage in a moderate amount of exercise – and this, without sacrificing weight gain.

Conclusions

Our results suggest that the ABA procedure in Long-Evans male rats results in hyperactivity and an inability to adequately increase food intake, resulting in weight loss. We found that the ABA effect was so robust under a 1 hour/day feeding schedule that rats could not survive for longer than 7 days. It would thus be relevant to assess whether the ABA effect could occur more gradually if the feeding window were increased to 90 minutes. An important limitation of the present experiment was the absence of a sedentary control group which would aid in determining the presence of an anorectic effect during the ABA phase. In addition, the limited sample size may have made it difficult to obtain statistically significant findings. The

following experiment will be aimed, in part, at addressing these limitations by increasing the feeding window, including a sedentary control group, and increasing sample size.

APPENDIX 3: THE RELATIONSHIP BETWEEN ABA SUSCEPTIBILITY AND PATTERNS OF RUNNING WHEEL ACTIVITY

Method

Subjects

Six female Sprague Dawley rats (125-150 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec). As in previous experiments, rats were kept on a 12:12 hr reverse light/dark cycle and their body weight, food intake, and water intake were monitored daily.

Apparatus

Running Wheel Cages. See “General Methodology” section.

Procedure

Acclimation Phase. Upon arrival, rats were individually housed in a colony room and allowed to acclimate for 5 days. During this phase, lights went off at 9 a.m. and on again at 9 p.m.

Entrainment to New Light Schedule. Following the 5 days of acclimation in the animal care facility, rats were moved to running wheel cages where they began entrainment to the new light schedule in which lights turned off at 7 p.m. and on again at 7 a.m. To train rats to eat during the light hours (in order to later dissociate between PPA and nocturnal activity (NA), rats were given *ad libitum* access to food for 8 hours from 9 a.m. to 5 p.m. (ZT 2 to 8). This phase lasted 10 days.

ABA Phase. The ABA phase started after the 10-day entrainment phase. Food was removed at 1:30 p.m. (ZT 6.5). On the following days, rats had access to food for 1.5 hr/day between 12 p.m. and 1:30 p.m. (ZT 5 to 6.5). The experiment was terminated after 6 days of ABA.

Statistical Analysis

A series of bivariate Pearson correlations were used to explore the relation between ABA susceptibility and different types of running wheel activity. Total change in body weight during the ABA phase was used as a representation of ABA susceptibility with more weight loss

indicating more susceptibility. Four types of activity on the final day of ABA were examined. FAA represented the average wheel rotations (per 10-min bin) in the 5 hours before feeding (ZT 0 to 5). PPA represented the average wheel rotations (per 10-min bin) during the 5 h 30 min following feeding (ZT 6.5 to 12). Nocturnal activity (NA) represented the average wheel rotations (per 10-min bin) during the 12 hours of the dark phase (ZT 12 to 24). Finally, feeding activity (FA) represented the average wheel rotations (per 10-min bin) during the 1.5- feeding period (ZT 5 to 6.5).

Results

There was a statistically significant positive correlation between FAA and PPA indicating that higher levels of running wheel activity during the hours following feeding were associated with higher running wheel activity prior to feeding ($r(4) = .82, p = .047$). Importantly, we observed that there was no relationship between FAA and weight loss during ABA ($r(4) = .57, p = .241$) while higher PPA activity seemed to be associated with more weight loss during ABA though this did not reach statistical significance ($r(4) = .78, p = .065$). See Table S1 for correlation matrix.

Table A3.1

Correlation Matrix for Weight Loss and Wheel Activity

		Weight Loss	FAA	FA	PPA	NA
Weight Loss		-				
FAA		.566	-			
FA		-.235	.195	-		
PPA		.783 ⁺	.816 [*]	.290	-	
NA		-.407	-.040	.186	-.450	-
	<i>M</i>	16.83	115.10	29.91	144.56	48.09
	<i>SD</i>	9.83	59.09	13.46	109.52	43.09

Note. $N = 6$.

^{*} $p < .05$. ⁺ $p < 1.00$

APPENDIX 4: THE EFFECT OF INHIBITING THE PRELIMBIC CORTEX USING DREADDs IN ABA

Method

Subjects

Twenty-four female Sprague Dawley rats (125-150 g) were purchased from Charles River Laboratories (Saint-Constant, Quebec). Twelve rats were used in supplemental experiment 4.1 and the remaining 12 were used in supplemental experiment 4.2. As in previous experiments, rats were kept on a 12:12 hr reverse light/dark cycle and their body weight, food intake, and water intake were monitored daily at ZT 11-12 throughout the experiments. Upon arrival, rats were individually housed in a colony room and allowed to acclimate for 7 days after which they received surgery and were relocated into running wheel cages. With the exception of the restriction phase, rats had *ad libitum* access to both food and water throughout the experiment.

Apparatus

Running Wheel Cages. See “General Methodology” section.

Drugs

In supplemental experiment 4.1, clozapine (Adooq Bioscience, Irvine, CA) was dissolved in 25% (wt/vol) dimethyl sulfoxide (DMSO) to a concentration of 1.0 mg/ml. This stock solution was further diluted with sterile saline based on the bodyweight of each individual rat in order to yield 0.1 mg/kg/infusion. Vehicle was 25% (vol/vol) DMSO in sterile saline.

In supplemental experiment 4.2, Clozapine-*N*-Oxide (CNO; Contribution from the National Institute on Drug Abuse) was dissolved in 5% DMSO and 95% sterile saline solution to a concentration of 100 mM. Vehicle was 5% (vol/vol) DMSO in 95% sterile saline solution.

Procedure: Supplemental Experiment 4.1. Prelimbic inhibition using Clozapine

Stereotaxic viral surgery. Following 7 days of acclimation in the animal care facility, rats underwent viral surgery. Rats were anaesthetised in an induction chamber with 3-4% isoflurane and maintained on 1-3% throughout the surgery. Rats were subcutaneously injected with 2 ml 0.9 % saline for hydration, 0.1 ml/kg atropine to control mucus build up, and 450 000 IU/rat penicillin to prevent infection. Rats received a bilateral microinjection of 0.75 µl of viral

vector (AAV8-hSyn-hM4D(Gi)-mCherry, lot #474) into the PL (1.60 AP, \pm .0.70 ML, -3.20 DV relative to Bregma) at a rate of 0.1 μ l/min. The injector was left in place for 10 min. Once removed, antibiotic ointment (Polysporin) was placed on the sutured area to reduce the risk of infection. Finally, rats received an additional 2 ml (sc) of 0.9 % saline for hydration and 2 mg/kg (sc) of Ketaprofen for pain relief. The subcutaneous Ketaprofen injections were repeated 24 hr and 48 hr post operation.

Wheel habituation phase. Following surgery, rats were transferred from the animal care facility to the individual running wheel cages where they were permanently housed for the remainder of the experiment. During this phase, all rats had *ad libitum* food and water and continuous access to the running wheel. This phase lasted 17 days. During the last two days of this phase, rats received a daily injection of 0.2 ml (ip) saline in an effort to habituate the rats to the injection procedure and minimize the impact of stress on running wheel activity. At the end of this phase, a median split of baseline running wheel activity was used to separate rats into resilient and susceptible groups. Each group was further divided into PL inhibition (CLZ) and vehicle control (VEH) groups by matching levels of food intake and running wheel activity.

ABA phase. Food was removed at ZT 13. On the following days, rats had access to food for 1 hr/day between ZT 12-13. During the ABA phase, rats received a daily ip injection of CLZ or VEH prior to feeding, resulting in PL inhibition for approximately 2 hr post-injection for rats in the CLZ condition. The restriction phase was terminated when rats reached the starvation criterion (weight loss of 25% of initial body weight).

Procedure: Supplemental Experiment 4.2. Prelimbic inhibition using Clozapine-N-Oxide

Stereotaxic viral surgery. Following a 7-day acclimation in the animal care facility, rats underwent viral surgery as described above for experiment 3.2.1 with the exception that PL coordinates were adjusted to 3.20 AP, \pm 0.70 ML, and -3.60 DV relative to Bregma as upward diffusion of the DREADDs into the cingulate cortex was noted in some of the previous placements.

Wheel habituation phase. Following surgery, rats were transferred from the animal care facility to the individual running wheel cages where they were permanently housed for the remainder of the experiment. During this phase, all rats had *ad libitum* food and water and

continuous access to the running wheel. This phase lasted 24 days. At the end of this phase, a median split of baseline running wheel activity was used to separate rats into resilient and susceptible groups. Each group was further divided into PL inhibition (CNO) and vehicle control (VEH) groups by matching levels of food intake and running wheel activity.

Osmotic mini-pump surgery. Following the 24 days of wheel habituation, rats underwent minipump surgery in which they were implanted with subcutaneous osmotic minipumps (Model 2002; Alzet, Cupertino, CA) filled with CNO or VEH solutions delivered chronically at a rate of 0.47 μ l/hr over 14 days. Rats were anaesthetised in an induction chamber with 3-4% isoflurane and maintained on 1-3% isoflurane throughout the surgery. Rats were injected with 2 ml (sc) 0.9 % of saline for hydration and 2 mg/kg (sc) Ketapofen for pain relief. A half-inch incision was made between scapulae to allow for insertion of the minipump and was closed using silk sutures. Polysporin was applied to the sutures to prevent infections.

Recovery. Rats were allowed to recover from the minipump surgery for 4 days. During this time, they remained in their wheel cages and had continuous access to food, water, and the running wheel.

ABA phase. Food was removed at ZT 13. On the following days, rats had access to food for 1 hr/day between ZT 12-13. After 3 days of ABA, the feeding period was reduced to 45 min out of concern that rats were not displaying the typical hyperactivity. The restriction phase was terminated when rats reached the starvation criterion (weight loss of 25% of initial body weight).

Statistical Analysis

Three 3-way mixed ANOVAs were used to examine the effects of OLZ on the following dependent variables: running wheel activity, food intake, and change in body weight. The between-subjects factors were *trait* (resilient vs. susceptible) and *treatment* (VEH vs. CLZ). The within-subjects factor was *phase* (habituation phase vs. ABA phase). The habituation phase was represented by the average of the dependent variable of interest across the last 5 days of this phase while the ABA phase was represented by the average of the dependent variable of interest across the first 5 days of this phase. Partial eta squared was used as a measure of effect size.

Results

Supplemental Experiment 4.1: Effect of prelimbic inhibition via clozapine on ABA development

Running wheel activity. Running wheel activity during the habituation and ABA phases can be seen in Figure A4.1A. Overall, susceptible rats ran significantly more than resilient rats (*trait*: $F(1,8) = 14.28, p = .005, \eta_p^2 = 0.64$). Across all rats, however, running wheel activity did not increase during the ABA phase, relative to the habituation phase (*phase*: $F(1,8) = 1.86, p = .210, \eta_p^2 = 0.19$). Furthermore, there was no overall effect of treatment condition (VEH vs CLZ) on running activity (*treatment*: $F(1,8) = 1.94, p = .202, \eta_p^2 = 0.20$). None of the interactions were statistically significant (*phase x trait*: $F(1,8) = 0.12, p = .742, \eta_p^2 = 0.01$; *phase x treatment*: $F(1,8) < 0.01, p = .987, \eta_p^2 < 0.01$; *trait x treatment*: $F(1,8) = 1.01, p = .345, \eta_p^2 = 0.11$; *phase x trait x treatment*: $F(1,8) = 0.05, p = .820, \eta_p^2 = 0.01$).

Food intake. As can be seen in Figure A4.1B, there was a statistically significant decrease in food intake during the ABA phase relative to the habituation phase (*phase*: $F(1,8) = 469.57, p < .001, \eta_p^2 = 0.98$). There was no difference in food intake between resilient and susceptible rats (*trait*: $F(1,8) = 0.90, p = .371, \eta_p^2 = 0.10$) and no difference between VEH rats and CLZ rats (*treatment*: $F(1,8) < 0.01, p = .974, \eta_p^2 < 0.01$). None of the interactions were statistically significant (*phase x trait*: $F(1,8) = 0.58, p = .468, \eta_p^2 = 0.07$; *phase x treatment*: $F(1,8) = 0.32, p = .590, \eta_p^2 = 0.04$; *trait x treatment*: $F(1,8) = 1.49, p = .258, \eta_p^2 = 0.16$; *phase x trait x treatment*: $F(1,8) < 0.01, p = .969, \eta_p^2 < 0.01$).

Change in body weight. Change in body weight during the habituation and ABA phases can be seen in Figure A4.1C. Overall, susceptible rats lost significantly more weight than resilient rats (*trait*: $F(1,8) = 7.69, p = .024, \eta_p^2 = 0.49$). Rats lost more weight during the ABA phase compared to the habituation phase (*phase*: $F(1,8) = 220.98, p < .001, \eta_p^2 = 0.97$). There was no overall effect of treatment condition (VEH vs CLZ) on change in body weight (*treatment*: $F(1,8) = 0.05, p = .830, \eta_p^2 = 0.01$). None of the interactions were statistically significant (*phase x trait*: $F(1,8) = 0.55, p = .479, \eta_p^2 = 0.07$; *phase x treatment*: $F(1,8) = 0.02, p = .891, \eta_p^2 < 0.01$; *trait x treatment*: $F(1,8) = 0.01, p = .914, \eta_p^2 < 0.01$; *phase x trait x treatment*: $F(1,8) = 0.91, p = .367, \eta_p^2 = 0.10$).

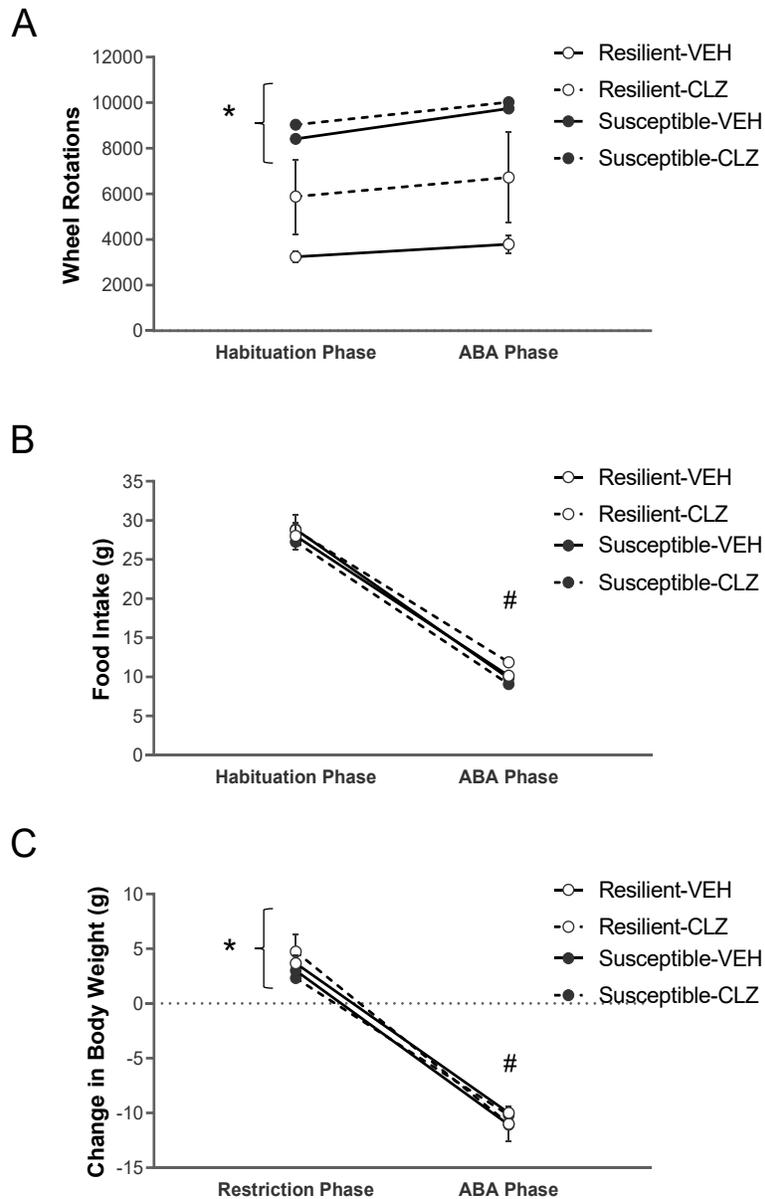


Figure A4.1. The effect of prelimbic inhibition using clozapine on ABA in resilient-VEH ($n = 3$), resilient-CLZ ($n = 3$), susceptible-VEH ($n = 3$), and susceptible-CLZ ($n = 3$) rats. (A) Mean running wheel activity during the habituation phase and ABA phase. * $p = .005$, main effect of *trait*. (B) Mean food intake during the habituation phase and ABA phase. # $p < .001$, main effect of *phase*. (C) Mean change in body weight during the habituation phase and ABA phase. * $p = .024$, main effect of *trait*. # $p < .001$, main effect of *phase*.

Supplemental Experiment 4.2: Effect of chronic prelimbic inhibition via CNO on ABA development

Running wheel activity. Running wheel activity during the habituation and ABA phases can be seen in Figure A4.2A. Overall, susceptible rats ran significantly more than resilient rats (*trait*: $F(1,5) = 22.66, p = .005, \eta_p^2 = 0.82$). While running wheel activity, across all rats, did not increase from the habituation phase to the ABA phase (*phase*: $F(1,5) = 5.95, p = .059, \eta_p^2 = 0.54$) an increase from habituation to ABA was observed in susceptible rats (*phase x trait*: $F(1,5) = 8.88, p = .031, \eta_p^2 = 0.64$). There was no effect of treatment (CNO vs. VEH) on running wheel activity (*treatment*: $F(1,5) = 0.07, p = .808, \eta_p^2 = 0.01$) and there were no other significant interactions (*phase x treatment*: $F(1,5) = 0.40, p = .556, \eta_p^2 = 0.7$; *trait x treatment*: $F(1,5) = 0.06, p = .819, \eta_p^2 = 0.11$; *phase x trait x treatment*: $F(1,5) = 0.05, p = .835, \eta_p^2 = 0.01$).

Food intake. As can be seen in Figure A4.2B, there was a statistically significant decrease in food intake during the ABA phase relative to the habituation phase (*phase*: $F(1,5) = 387.36, p < .001, \eta_p^2 = 0.99$). There was no difference in food intake between resilient and susceptible rats (*trait*: $F(1,5) = 0.16, p = .748, \eta_p^2 = 0.02$) and no difference between VEH rats and CLZ rats (*treatment*: $F(1,5) = 0.19, p = .682, \eta_p^2 = 0.04$). None of the interactions were statistically significant (*phase x trait*: $F(1,5) = 0.0.67, p = .451, \eta_p^2 = 0.12$; *phase x treatment*: $F(1,5) = 0.55, p = .492, \eta_p^2 = 0.10$; *trait x treatment*: $F(1,5) = 2.38, p = .184, \eta_p^2 = 0.32$; *phase x trait x treatment*: $F(1,5) = 0.05, p = .835, \eta_p^2 = 0.01$).

Change in body weight. Change in body weight during the habituation and ABA phases can be seen in Figure A4.2C. Overall, susceptible rats lost significantly more weight than resilient rats (*trait*: $F(1,5) = 6.90, p = .047, \eta_p^2 = 0.58$). Rats lost more weight during the ABA phase compared to the habituation phase (*phase*: $F(1,5) = 199.02, p < .001, \eta_p^2 = 0.98$) and this difference appeared to be most present in susceptible rats though the *phase x trait* interaction was trending only (*phase x trait*: $F(1,5) = 4.54, p = .086, \eta_p^2 = 0.48$). There was no overall effect of treatment condition (VEH vs CLZ) on change in body weight (*treatment*: $F(1,5) = 0.01, p = .982, \eta_p^2 < 0.01$). The other interactions were not statistically significant (*phase x treatment*: $F(1,5) = 1.55, p = .269, \eta_p^2 = 0.24$; *trait x treatment*: $F(1,5) = 0.24, p = .645, \eta_p^2 = 0.05$; *phase x trait x treatment*: $F(1,5) = 0.88, p = .391, \eta_p^2 = 0.15$).

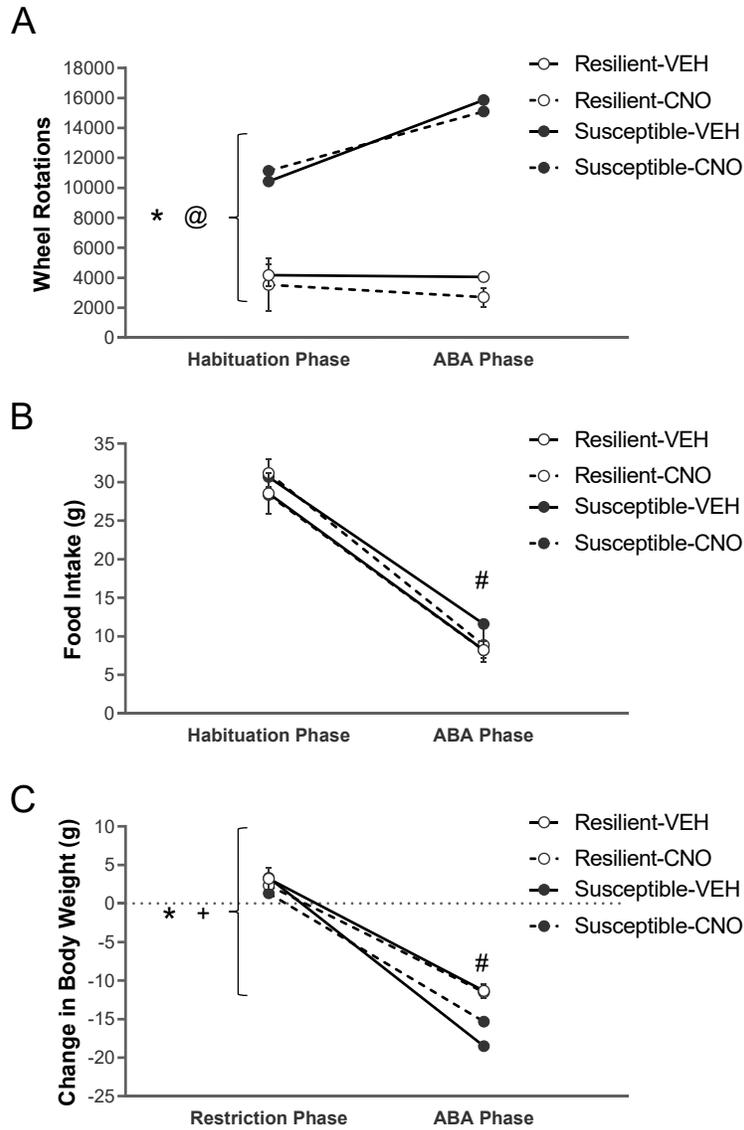


Figure A4.2. The effect of prelimbic inhibition using CNO on ABA in resilient-VEH ($n = 3$), resilient-CNO ($n = 2$), susceptible-VEH ($n = 2$), and susceptible-CLZ ($n = 2$) rats. (A) Mean running wheel activity during the habituation phase and ABA phase. * $p = .005$, main effect of *trait*. @ $p = .031$, *phase x trait* interaction. (B) Mean food intake during the habituation phase and ABA phase. # $p < .001$, main effect of *phase*. (C) Mean change in body weight during the habituation phase and ABA phase. * $p = .047$, main effect of *trait*. # $p < .001$, main effect of *phase*. + $p = .086$, *phase x trait* interaction.