Therapeutic Effects of Flibanserin in Rat Activity-Based Anorexia

by

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Abstract

Anorexia nervosa (AN) is a complex disorder with high morbidity and mortality. Given that multi-modal rehabilitation programs are the only effective AN treatment, the discovery of new drug treatments is essential. Core to researching new treatment options for AN is the use of animal models, specifically, activity-based anorexia (ABA). ABA reproduces three of the core symptoms of AN: caloric restriction, increased exercise, and rapid weight loss. Flibanserin is a drug that increases sexual activity in women, and we hypothesized that it might increase another rewarding motivated activity, eating, in juvenile female ABA rats. Our findings indicate that flibanserin treatment reduced weight loss by decreasing hyperactivity and increasing food intake. Further examination of the complex pharmacological profile of FLIB is required to understand the pharmacological mode of action underlying the behavioral changes in ABA, however this research provides clinically relevant evidence that flibanserin may be effective in combatting ABA symptoms.
Introduction

Anorexia Nervosa

Eating disorders (EDs), including Anorexia Nervosa (AN), Atypical Anorexia, Bulimia Nervosa (BN), and residual feeding disorders (eating disorders not otherwise specified; EDNOS) are a major health concern. The AN patient death rate is nine times higher than age-matched peers, and in long-term follow up studies, up to 18% of deaths were attributed to AN (Arcelus et al, 2011; Khalsa et al, 2017; Mehler et al, 2022). The incidence of AN among those under 15 years of age is increasing, and recent statistics estimate a lifetime prevalence of 4% in females and 0.3% in males (Suokas et al., 2013; van Eeden, van Hoeken & Hoek 2021).

The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders characterizes anorexia by “distorted body image and excessive dieting that leads to severe weight loss with a pathological fear of becoming fat”. In addition, patients often have body dysmorphic disorder (BDD), socially isolate themselves, deny their state of starvation, and resist treatment despite their emaciated state. A curious symptom that afflicts ED patients is sexual dysfunction, specifically, the loss of sexual desire. A 2010 study published in the International Journal of Eating Disorders that examined sexual function in 242 female ED patients found that 75% of those patients with AN, versus 39% of BN and 45% of EDNOS sufferers, experienced a loss of libido, and the percentage of sexual dysfunction experienced in any ED was higher than that in a normative sample (Pinheiro et al., 2010). 80% of AN patients partake in excessive, strenuous exercise, a symptom described as having “addiction”-like properties and a key attribute of the
disorder (Klein et al., 2004). Sufferers exhibit dramatic weight loss due to self-starvation and exercise, and consequently experience various health problems associated with being severely underweight.

Progress has been made towards discovering the biological underpinnings of EDs, however they continue to be characterized as ailments with largely psycho-socio-cultural aetiologies. The first modern case of AN was identified by Sir William Gull in 1873 and published in the Lancet in 1888, though clinical manifestations of eating disorders that correspond with DSM-IV-TR diagnostic criteria are documented in Mediterranean texts from the first through seventh century AD (Tsagkaris et al, 2022). While the media and the Western obsession with thinness and BMI have a clear causal role in body dissatisfaction and EDs, a proliferation of bio-psychiatric scientist-practitioners see this contention as a diversion from the need to acknowledge that EDs are severe, self-sustaining psychiatric illnesses with genetic, biochemical bases (Levine and Maine, 2010). The distraction away from biological factors is reflected not only in the stigma associated with EDs (that they are a choice) but also in the low success rates of AN treatment strategies.

A survival analysis of patients with anorexia following completion of a specialized in-patient treatment program at the Toronto General Hospital reported a relapse rate of 35% (Carter et al., 2004). A systematic review from 2017 of AN relapse rates following in-patient treatment concluded that the average relapse rate is between 9-52%, which spikes within the first year, then gradually increases over time (Khalsa et al, 2017). Relapse prevention programs, when administered following in-patient care, reduced relapse rates to 30% (Berends et al, 2016). Anorexia nervosa is a chronic, often
remitting-relapsing disorder, and although 50-70% of those afflicted with AN will recover, it can take up to 10 years (Evans et al., 2006). These statistics highlight the need for standardized definitions of recovery, remission, and relapse, as well as the urgent need for novel treatment strategies.

Rehabilitation programs span months and include extensive collaboration between mental health care professionals, a nutritionist or dietician and a medical doctor who specializes in eating disorders, and thus the cost to the health care system, and therefore to society, is extremely high. Given that AN is under-detected and under-treated (Simon, Schmidt, & Pilling, 2005), the economic burden imposed by EDs will likely rise in the future. Although some progress has been made towards ascertaining the neurobiological underpinnings of EDs, many unknowns remain. This has hindered the progress of successful treatment interventions as there are currently no effective pharmacological treatments for AN. High dose SSRIs have historically been prescribed to patients with AN to treat obsessive-compulsive symptoms related to food and in cases of co-morbid depression or anxiety symptoms, however they appear to have minimal effect on weight gain, so the efficacy and modality of these treatments are disputed (Claudino et al, 2006; Sebaaly et al, 2013; Reas & Grilo, 2021).

A biological basis for AN is supported by its heritability, which is estimated to be 70% (Ramoz, 2013). First-degree relatives of those who have suffered AN are 11 times more likely to have AN than the relatives of unaffected individuals (Strober et al., 2000). Although AN is likely influenced by complex interactions between genetic variants, environmental and social factors, there is compelling evidence that implicates the reward system in the pathophysiology of AN.
Abnormal Eating is Associated with Dysregulation of Reward Circuits

The mesolimbic reward circuit involves glutamatergic input from the cortex to the nucleus accumbens (NAc) of the ventral striatum, and dopaminergic projections from cell bodies in the ventral tegmental area (VTA); this release of dopamine (DA) onto the NAc is integral to the drive aspect of reward and is thought to motivate consummatory behaviors, including food intake. Reward is also mediated through additional brain structures. The VTA projects to the amygdala and hippocampus, which helps reinforce salient memories. Connections between the VTA and the arcuate nucleus of the hypothalamus modulate appetite/satiety. The VTA also projects to the striatum, prefrontal cortex (PFC) and anterior cingulate cortex (ACC), all of which are involved in reinforcing learning and behaviors (Kelley & Berridge, 2002).

Anorexia nervosa has been hypothesized to develop in part because food restriction is initially perceived as rewarding and is then maintained through reinforcement of starvation-induced reward (Monteleone and Maj, 2013). Another hypothesis is that dieting and exercise initially increase DA, which allows these weight-loss behaviors to become established, but chronic food restriction leads to decreased DA and the entrenchment of AN behaviors (Beeler & Burghardt, 2022). Several behavioural manifestations of low DA levels are expressed in AN, like obsessive-compulsiveness, risk taking and anhedonia. It is possible that individuals with DA abnormalities restrict food as a coping mechanism, as food restriction has been shown to relieve anhedonic states. More specifically, improvements in anhedonia and mood are positively correlated with the magnitude of weight lost (Morris et al., 2012). Anhedonia in AN is supported by the fact that both neuroanatomical and activational abnormalities have been shown across
the reward circuit in acute and individuals who have recovered from AN (Monteleone and Maj, 2013).

A fMRI study by Holsen et al (2012) identified hypoactivation of reward-related brain regions including the ventral striatum in patients with anorexia when shown images of food, compared with controls. Similarly, Redgrave et al (2008) showed hyperactivation of the same reward regions in underweight AN patients upon processing starvation-linked stimuli. The degree of malnutrition in patients with AN corresponds with their scores on depression, anxiety, and obsessive-compulsiveness measures (Pollice, Kaye, Greenk & Weltzin, 1997) and thus the neurological abnormalities seen in AN patients have been hypothesized to be attributed to starvation (Pollice et al, 1997; Kaye et al, 2009). As there are no longitudinal studies examining individuals prior to the onset of AN, biological or behavioral abnormalities may be pre-existing and predisposing to AN. Although several biological aberrations seen in AN ameliorate with weight gain, DA system dysfunctions do not improve after recovery. Specifically, weight-recovered subjects with anorexia have lower levels of DA than controls and show increased inhibitory DA receptor binding in the striatum (Kaye et al, 2009). It follows that lower DA levels and activity in the PFC and ACC-NAc projection are seen in chronic and recovered AN patients (Mühlau et al, 2007; Su et al, 2021; Beeler & Burghardt, 2022). Activity in the dorsolateral PFC (dIPFC) was measured during a reward anticipation task where participants were presented with button-pressing tasks for monetary gain in an MRI. Chronic and patients recovered from AN showed increased activity in their dIPFC throughout the task, compared to controls. During both the reward-anticipation and reward-feedback phases of the trials, recovered AN patients displayed significantly higher activation in their dIPFC, an area core to
cognitive control over primary drives, including food and sex choices (Ehrlich et al, 2015).

Single nucleotide polymorphisms (SNPs) associated with AN have been found in the DRD2 and DRD4 genes, both of which code for inhibitory D2-like receptors (Rask-Andersen et al., 2010); when bound to DA or DA agonists they inhibit adenylyl cyclase thus downregulating the intracellular cAMP signalling pathway (Rask-Andersen et al, 2010). Dopamine D2 receptors are found on postsynaptic DA neurons concentrated in the mesocorticolimbic reward pathway and on astrocytes. Dopamine D2 receptor signalling is involved in hormone production, locomotion, appetitive behavior including substance abuse, and inflammation signalling. The functional polymorphism of human DRD2, TaqIA, involves a change in a single C/T nucleotide, resulting in A1 (T) and A2 (C) alleles. Not only has this polymorphism been associated with reward-driven behaviors including substance abuse and overeating, but it has also been associated with food preference and body mass. In fact, PET imaging studies in obese individuals have shown a proportional relationship between body weight and decreased density of striatal DA receptors (Volkow et al, 2003; Volkow et al, 2008). Carrying the A1 TaqIA allele results in reduced striatal activity following the viewing or consumption of palatable food, a preference for carbohydrates, and a greater risk of obesity (Volkow et al, 2003; Volkow et al, 2008; Stice et al, 2008; Stice et al, 2010; Cameron et al, 2013). Given the relationship between DRD2, food preference and body weight, further investigation as a potential treatment target for eating disorders is warranted.

The progression of food restriction and weight loss is enhanced by many factors that affect both feeding and reward pathways. A negative energy balance predicts high
ghrelin levels and low leptin levels, both of which enhance reward. Corresponding leptin and ghrelin tones are typical of AN, and Monteleone and colleagues hypothesize that these feeding peptide levels facilitate and maintain rewarding behaviours (Monteleone and Maj, 2013). Specifically, low leptin and high ghrelin reinforce food restriction by increasing activity in the mesolimbic dopaminergic reward pathway. Increased reward signalling due to hunger peptide levels is a possible mechanism through which patients’ control of overeating is perceived as rewarding, furthering AN progression (Monteleone and Maj, 2013). Energy deficiency also leads to a cascade of hormonal events that results in the suppression of the hypothalamic-pituitary-ovarian axis to conserve energy: high ghrelin, low leptin, and other metabolic changes result in decreased GnRH release, low estrogen, progesterone and consequently amenorrhea in AN (Miller, 2013). Interestingly, women with menstrual cycle aberrations who exercise often also restrict food intake, are fixated on losing weight, and exhibit disordered eating behavior (De Souza et al., 2007; Gibbs et al., 2011). In fact, the energy deficiency produced by restricting food intake and/or exercising, which is the case in individuals with AN, stimulates compensatory mechanisms that include weight loss (Sundgot-Borgen, 1994; Beals & Manore, 2001; McLean & Barr, 2003; De Souza & Williams, 2004).

Although food restricting behaviour and weight loss may counteract reduced capacity to experience reward, AN patients continue to starve themselves despite achieving dangerously low weights. The inability of AN patients to perceive life-threatening consequences of being severely underweight is another symptom characterized by low DA and altered reward processing. Cognitive flexibility requires a healthy balance between D1 and D2-like receptors in the PFC, and is dependent on DA
levels, which are intrinsically low in the PFC of both acute and weight-restored AN patients (Floresco, 2013). The human DRD4 gene is highly polymorphic and linked to substance abuse, mood disorders, ADHD and eating disorders (McCracken et al, 2000). Dopamine D4 receptors are expressed on prefrontal cortex pyramidal and interneurons, throughout the limbic system and on medium spiny neurons in the striatum and NAc in the basal ganglia. Bound D4 inhibits glutamatergic pyramidal neurons in the frontal cortex. DRD4 knockout mice show a 64% increase in resting levels of glutamate in their striatum, indicating that prefrontal glutamate release modulates striatal glutamate transmission (Thomas, Grandy & Gerhardt, 2009).

Low DA levels in the NAc-ACC circuit second to D2/D4 receptor malfunctions in acute AN patients are hypothesized to lead to cognitive impairment with respect to decision-making, risk evaluation, and behavior flexibility, which may explain why AN behaviors become entrenched (Mühlau et al., 2007; Beeler & Burghardt, 2022). The ACC is an area of the brain involved in cognition and emotional processing, with outputs that help motivate goal-directed attention and behavior. The ACC is atrophied in AN patients, which is hypothesized to be responsible for the body image distortion, abnormal reward processing and cognitive control of appetite seen in AN (Mühlau et al., 2007; Kaye, Fudge & Paulus, 2009). A whole brain meta-analysis examining structural and functional changes in AN, including over 1000 AN brains, revealed decreased gray matter volume in the ACC, posterior cingulate cortex, and in the left middle occipital gyrus extending to the left inferior parietal lobe (Su et al, 2021). These areas were also seen to have lower resting-state functional activity, along with an increase in activity in the right parahippocampal gyrus, compared to healthy controls (Su et al, 2021).
Acute AN patients and individuals with substance use disorders show chronically increased DA in the mesolimbic reward pathway, which only normalizes following detoxification in the case of substance use and weight gain in AN (Avena and Bocarsly, 2012). Lower levels of DA (compared to controls) are not exclusively seen in recovered AN patients: brains of individuals with substance use disorders also show reduced DA after long-term drug use (Nemoda et al., 2011). Dopamine in both the NAc and ACC is reduced in individuals suffering from alcohol-use disorders and long-term substance use disorders, characterized in imaging studies as reduced functional activity (Kuhn et al., 2011), similar to AN. Despite over 50 years of research, the etiology of AN remains poorly understood.

**Sex & Food**

Like eating, sexual activity is required for species survival, and thus produces and reinforces natural reward mediated by the mesolimbic reward circuit. Sexual arousal is mediated primarily by dopaminergic innervation of the mesolimbic and hypothalamic circuitry (Pfaus, 2009). Two opposing systems control sexual desire: excitation and inhibition. The excitation phase in female rodents is mediated by estrogen- and progesterone-mediated upregulation of DA, norepinephrine (NE), oxytocin and melanocortin in the hypothalamus and limbic system (Pfaus, 2009). Mesolimbic projections from the VTA to the amygdala, NAc, and ACC, mesocortical projections to the medial prefrontal cortex (mPFC), and nigrostriatal projections from the substantia nigra to the striatum are active during sexual arousal (Pfaus, 2009). During sexual satiety,
5-HT modulates descending inhibitory projections from the PFC, inhibiting mesolimbic and hypothalamic DA release (Pfaus, 2009) and therefore reward.

In many species, hormones and neurotransmitters that mediate hunger and satiety are intrinsically related to sexual behavior. Leptin inhibits food intake and increases energy expenditure, which is required for reproduction. The hormonal relationship between food and sex, however, is more complicated than this. Specifically, the balance of leptin and Neuropeptide Y (NPY) can predict whether rats will choose to consume sucrose or mate with an estrous female. A study done by Ammar and colleagues demonstrated that when given the choice between an estrous female and sucrose, a well-fed rat would choose the female every time. When rats are treated with leptin, they choose females over sucrose, but if these rats are treated with NPY, they choose sucrose over females (Ammar et al., 2000). Other studies have also shown that NPY treatment directs attention of hamsters toward food (Schneider et al., 2013). Ammar et al hypothesized that NPY may not only be orexigenic, but specifically diverts attention toward food, while leptin reduces food intake and directs attention away from food - toward other stimuli. Schneider furthered this and postulated that leptin and NPY control appetite and appetitive sexual behavior (Schneider et al., 2013).

There are several neural processes that are shared by eating and sex. One link between food and sex circuitry is the effect of 5-HT on motivated behaviors: hypothalamic 5-HT inhibits sexual behavior and decreases feeding (Blundell, 1986). Food deprivation in rodents results in increased 5-HT tone in the lateral, ventromedial and paraventricular nuclei of the hypothalamus (Schwartz et al, 1989; Schwartz et al, 1990; Fetissov et al, 2000; Tachibana et al, 2001). Increased hypothalamic 5-HT inhibits
the release of DA from the NAc, thus inhibiting reward activation and blunting feeding and sexual arousal (Pfaus, 2009; Verhagen et al., 2009). It follows that medications which increase extracellular hypothalamic 5-HT suppress appetite (Hoebel et al, 1989; Paez & Leibowitz, 1993) and decrease sexual behavior (Meyerson, 1964; Blundell, 1986).

**Flibanserin**

Flibanserin (FLIB) is a drug that increases appetitive sexual behaviors, presumably through decreasing prefrontal, hypothalamic and mesolimbic 5-HT and increasing DA and NE in the PFC (Borsini et al, 2002; Allers et al, 2010; Stahl et al, 2011). Flibanserin was developed by Boehringer Ingelheim Pharma, and advanced to clinical trials as an alternative, fast-acting antidepressant in 2004. Although it did not significantly improve depressive symptoms, researchers noticed an increase in self-reported sex drive, pleasure from sex, and number of pleasurable sexual encounters from women who participated in the clinical trial. In August 2015 FLIB was approved by the United States food and drug administration for treatment of hyposexual desire disorder, or low libido in premenopausal women. Flibanserin is the first drug to target women’s libido, and acts on excitatory dopaminergic, noradrenergic, and inhibitory serotonergic tone in pathways involved with appetitive motivation (Allers et al., 2010).

Flibanserin is a dual serotonin receptor agonist-antagonist: it agonizes postsynaptic 5-HT$_{1A}$ receptors and antagonizes 5-HT$_{2A}$ receptors. Flibanserin is also an agonist at DA D$_{4}$ receptors. Animal studies indicate that chronic FLIB preferentially activates 5-HT$_{1A}$ receptors in the prefrontal pyramidal neurons, which in turn leads to hormone secretion (including the release of oxytocin, prolactin, β-endorphin and growth
hormones) and an increase in DA release in the mPFC, striatum and hippocampus (Borsini et al., 2002; Stahl et al., 2011). Dopamine release in these areas is accompanied by 5-HT$_{1A}$ receptor agonism and 5-HT$_{2A}$ receptor antagonism: binding these receptors inhibits glutamate release from pyramidal PFC neurons, which, in turn, reduces GABA inhibition of DA neurons, leading to increased DA release into the PFC (Stahl et al., 2011). The FLIB-induced DA increase in the PFC is long lasting and is hypothesized to contribute to continual therapeutic actions on sexual desire (Stahl et al., 2011).

Flibanserin increases cfos expression in the arcuate nucleus, ventral tegmental area (VTA) and locus coeruleus, and increases catecholaminergic neuron density in the VTA (Gelez et al, 2013). In healthy female rodents, FLIB acts to increase NE and decrease 5-HT in the mPFC, medial preoptic area (mPOA) and NAc, while increasing DA in all areas but the NAc (Allers et al., 2010). Although drugs that increase DA tend to be stimulants and increase locomotor activity, in rats, FLIB does not appear to do this. Indeed, FLIB not only decreases motor activity, but it also blocks excessive locomotor activity induced by stimulants (Borsini et al., 1999; Borsini & Cesana, 2001; Ferger et al., 2010). Since FLIB does not increase DA in the NAc, it does not generate self-administration or place preference (Borsini & Cesana, 2001). Overall, FLIB appears to reduce cortical firing by stimulating postsynaptic 5-HT$_{1A}$ receptors and reduce the number of active neurons by stimulating D$_4$ receptors (Borsini et al, 2002). Animal studies demonstrate that acute FLIB administration at clinically relevant dosages (1-32 mg/kg/day) modulates monoamines, increasing baseline levels of DA and NE in the PFC and decreasing 5-HT levels in the PFC, NAc and hypothalamus.
Activity Based Anorexia

Animal models of eating disorders are critical to AN research as testing new treatments and observing how they affect the brain is not possible in humans. The activity-based anorexia (ABA) model was developed by Hall and Hanford in 1953 (Hall & Hanford, 1954) and remains the most widely utilized animal model of AN. The basic paradigm involves allowing rodents 22-24-hour access to a running wheel while restricting food intake to a daily 1-2-hour period (Schalla & Stengel, 2019). Hall and Hanford observed that rats subject to ABA conditions would starve and run themselves to death, unless the experimenter intervened, like what is seen in severe AN patients, even with medical intervention. Their original ABA research paper detailed the protocol in adult male rats, but only reported results for running behavior. Control rats running remained stable across 3 weeks. Rats that were food restricted ran, on average 6 times more than their base running rate, which increased over the 3 weeks. Given the age- and sex-specific onset of anorexia nervosa in adolescent girls, juvenile female rodents are now more commonly used for ABA research. Under ABA conditions, adolescent female rats run more than controls and lose weight quickly, but do not necessarily eat less than food-restricted controls. Thus, ABA symptoms in young female animals include rapid weight loss and hyperactivity (Dixon et al., 2003), loss of estrous, and development of hyperactivity within 1-2 weeks (Dixon et al., 2003). Rats subject to ABA conditions for extended periods of time may become hypothermic, develop stomach ulcers, and will eventually die – symptoms parallel to those seen in human anorexia patients.
Previous ABA Research

Previous ABA research supports the involvement of disruptions in DA as well as 5-HT signaling in AN. The first successful pharmacological intervention for ABA was discovered when male ABA rats showed recovery of food intake and reduced running when treated systemically with chlorpromazine, a DA antagonist (Routtenberg & Kuznesof, 1967). Similarly, when given a nonselective DA antagonist (cis-flupenthixol), female ABA rats show reduced activity, reduced weight loss and increased food intake (Verhagen et al., 2009a; Avena and Bocarsly, 2012). Eticlopride and Amisulpride, both D2 and D3 receptor antagonists, increased survival in the ABA model (Kleinotich et al, 2015). Olanzapine, an atypical antipsychotic and DA antagonist, caused reduced running in female ABA rats, but treatment did not help reduce weight loss (Hillebrand et al., 2005). Female mice who underwent maternal separation prior to ABA showed increased DA cells in their VTA and a greater density of 5-HT cells in their lateral hypothalamic area, as well as greater hyperactivity than non-separated ABA animals (Aspesi et al, 2021). Mild to moderate short-term stressors, like immobilization or foot shock, increase DA release in the NAc and mPFC, while chronic uncontrollable stressors, like maternal separation, inhibit DA release (Cabib & Puglisi-Allegra, 2012; Holly & Miczek, 2016; Baik, 2020). Taken together, this suggests that maternal separation stress primes animals to respond differently to future stressors, including ABA vulnerability, possibly by increasing VTA DA cells to adapt to low DA levels.

Several studies that focused on directly modifying DA levels and reward circuit activation were able to alter vulnerability and resilience to ABA. One study that examined resilience and vulnerability in ABA mice demonstrated that heterozygous DAT
knockdown using DA transporter-Cre recombinase mice showed higher DA peaks and DA was cleared more slowly from synapses across the striatum, and that the knockdown mice were more vulnerable to ABA. These hyperdopaminergic mice increased light-cycle running behavior more quickly than wild type mice, indicating that constantly increased striatal DA induces vulnerability by accelerating increases in daytime hyperactivity in response to food restriction (Beeler et al, 2020). Another study examining ABA development and recovery in rats used a dual viral activation strategy, allowing them to specifically depolarize dopaminergic neurons projecting from the VTA to the NAc upon injection of an activating compound, clozapine-n-oxide (CNO). Specifically, they injected one virus (CAV-2-CreEGFP from IGMM in France) into the NAc of rats, which was transported back to the VTA, and another virus (+M₃-mCherry, from UNC Vector Core, USA) directly into the VTA. The +M₃-mCherry virus contains the floxed inverted sequence of hM₃D(Gq)-mCherry, a G protein-coupled receptor, which reorients to allow DREADD receptor expression and binding in the presence of Cre (Foldi, Milton & Oldfield, 2017). The +M₃-mCherry is therefore only expressed in cells also infected with CAV-2-CreEGFP, which are isolated in the VTA (Foldi et al, 2017). Rats who were injected with CNO 30 minutes before food presentation throughout the ABA paradigm ate significantly more food and lost significantly less weight, despite running as much as controls (Foldi et al, 2017). Constant increases in ventral reward circuit DA likely infer vulnerability by enhancing the rewarding value of wheel running (as food, mates, and other stimuli are unavailable during the ABA paradigm), while increases immediately prior to food presentation infer resilience to ABA.
Food deprivation in rodents has been shown to increase 5-HT tone in the lateral, ventromedial and paraventricular nuclei (PVN) of the hypothalamus, reducing feeding and blunting reward response (Schwartz et al, 1989; Schwartz, Hernandes & Hoebel, 1990; Paez & Leibowitz, 1993). Medications that increase extracellular hypothalamic 5-HT suppress appetite and decrease sexual behavior (Blundell et al, 1986; Hoebel et al, 1989). Jean et al investigated the effects of agonizing 5-HT4 receptors in the NAc of food deprived and fed mice and found that directly stimulating these receptors reduced motivation to eat (inhibited food intake) and increased cocaine- and amphetamine-regulated transcript mRNA in the NAc in both fed and unfed states (Jean et al., 2007). A study measuring 5-HT, DA and their metabolites in ABA rats found that both DA and 5-HT release in the NAc was decreased in ABA rats, although DA was increased during the feeding period (Verhagen et al., 2009b). Serotonergic circuits may oppose rewarding appetitive DA pathways, as 5-HT is reduced in the nucleus accumbens (NAc) of ABA animals (Verhagen et al., 2009b) and supressing 5-HT using 8-OH-DPAT results in weight gain and reduced hyperactivity in ABA animals (Atchley and Eckel, 2006).

Stress also contributes to the development of ABA, and the nature of the paradigm design is stressful for the rodents. Chronic exposure to restricted feeding and exercise activates the HPA axis and results in excessive secretion of glucocorticoids (Rivest & Richard, 1990; Burden et al., 1993). These glucocorticoids, specifically corticosterone, modulate firing and DA synthesis in the VTA, in turn activating reward (Overton et al., 1996). Interestingly, chronic stress often precedes and contributes to the maintenance of both disordered eating and low libido.
Flibanserin in AN and ABA

It has been established that FLIB reduces locomotor activity, a core symptom of ABA and AN, and if FLIB acts to promote sexual desire, feeding (another motivated behavior) may also be increased through FLIB actions on DA and 5-HT. Indeed, there are proposed reward pathway deficits in the pathologies underlying both sexual desire disorder and anorexia nervosa (Janssen & Bancroft, 2006; Mühlau et al., 2007; Kaye et al., 2008; Berridge, 2008; Pfau, 2009; Allers et al., 2010; Stahl et al., 2011; Floresco, 2013). Hypoactive sexual desire is hypothesized to be caused by an imbalance between neurotransmitters that have excitatory versus inhibitory effects, specifically the balance between DA, NE, and 5-HT. If FLIB increases sexual desire by increasing DA in the PFC, and AN patients have low DA in the PFC (Floresco, 2013), it is possible that by increasing DA in the PFC, eating may increase. Since 75% of patients with AN experience sexual dysfunction (Pinheiro et al., 2010), helping rebalance DA and 5-HT may assist in remotivating both eating and sex drive.

If FLIB reduces 5-HT and regulates NE appetitive signaling in the hypothalamus, it may suppress food restriction and hyperactivity in ABA as 5-HT binding increases activity and reduces food intake (Clifton et al., 2000; Lee et al., 2002; Hewitt et al., 2002; Verhagen et al., 2009b). Increasing NE in the PVN induces a robust feeding response (Leibowitz, 1988), as well as increasing dopaminergic tone in the NAc in females (Parada et al, 1995; Baptista et al, 2002). Given that DA is reduced in AN, and FLIB increases DA, the current study examines if FLIB is effective in combating AN-related symptomology. If exercising and food restriction are used to compensate for reduced DA in the PFC and mesocorticolimbic reward pathway, we hypothesize that FLIB may
provide sufficient therapeutic relief through increases in excitatory DA and decreases in inhibitory 5-HT in the reward pathway.
Study Rationale, Objectives and Hypotheses

Study Rationale & Research Objectives

Anorexia nervosa is a severe, chronic eating disorder characterized by food restriction, excessive exercise, and rapid weight loss. Given that rehabilitation programs are currently the only effective treatment options for AN, further understanding of the neurobiological factors underlying the disorder are needed to facilitate potential new drug treatments. Flibanserin is a drug that increases appetitive motivation, acts as an alternative antidepressant both in humans and in rodents and is used for treatment of female sexual interest/arousal disorder. Notably, FLIB targets DA, NE, and 5-HT in the brain, all of which have been implicated in AN. Activity-based anorexia is the rodent model of AN, in which rodents who are both food restricted and provided an activity wheel rapidly lose weight. We proposed experiments that addressed the following specific objectives:

- To pilot the ABA protocol in juvenile female rats
- To investigate the therapeutic effects of FLIB on food intake, running activity and weight loss in ABA rats

Research Hypothesis

Given that DA is reduced in AN, and FLIB increases DA, we hypothesized that FLIB may be effective in combating AN-related behaviors/symptoms. Though the mechanism by which FLIB exerts its effects in ABA remains to be determined, it is possible that the known effects of FLIB on inhibitory/excitatory balance in the PFC may underlie the therapeutic effects, especially given the posited role for PFC
inhibitory/excitatory balance in AN. To test the hypothesized role of FLIB in combatting AN, we used an ABA model and hypothesized that FLIB will decrease running activity and weight loss. Given that AN disproportionately occurs in young females, we employed juvenile female rats as subjects.
Methods

Experimental Animals and Procedures

Activity-based anorexia is modelled in rodents by restricting their food intake and providing them wheel access; the experimental procedures were as follows. Fifty-five female Sprague-Dawley rats (5 weeks old) were obtained from Charles River Canada. Upon arrival, rats were immediately single housed in standard polypropylene cages and acclimatized for 72 hours, during which they had ad libitum access to food and water. They were maintained on a reverse 12-h hour light/dark cycle in a temperature-controlled environment (21°C +/- 1). Rats were pre-tested for wheel running behaviour to identify and exclude low-running rats from further experimental manipulation (see Figure 1 for timeline). Rats were then randomly assigned to 1 of 5 groups (see Table 1). For the duration of 5 days, weight, food intake, and wheel running (where applicable), were measured at the start of the dark cycle. All rats were weighed 60 minutes after the start of the dark cycle, with food provided to food-restricted rats for a 90-minute period, which began 90 minutes after the start of the dark cycle. Food was weighed at the start and end of the feeding period to determine food intake. For non-food restricted rats, food was weighted daily, 3 hours after the start of the dark cycle. Food-restricted animals were immediately euthanized if weight loss reached >35% of expected weight (compared to weight-matched non-food-restricted rats). All animal use procedures were approved by the Carleton University Committee for Animal Care, according to the guidelines set by the Canadian Council for the Use and Care of Animals in Research.
**Table 1. Group Summary Table.** Names of groups and their respective experimental conditions regarding food restriction, wheel access and drug treatment.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Food</th>
<th>Activity</th>
<th>Drug Treatment</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (CTRL)</td>
<td>Ad Lib</td>
<td>No Wheel</td>
<td>Vehicle</td>
<td>10</td>
</tr>
<tr>
<td>Wheel Access Only (WH)</td>
<td>Ad Lib</td>
<td>Wheel</td>
<td>Vehicle</td>
<td>10</td>
</tr>
<tr>
<td>Food Restriction Only (FR)</td>
<td>Food Restricted</td>
<td>No Wheel</td>
<td>Vehicle</td>
<td>10</td>
</tr>
<tr>
<td>ABA-Vehicle (ABA-V)</td>
<td>Food Restricted</td>
<td>Wheel</td>
<td>Vehicle</td>
<td>10</td>
</tr>
<tr>
<td>ABA-FLIB</td>
<td>Food Restricted</td>
<td>Wheel</td>
<td>Flibanserin</td>
<td>10</td>
</tr>
</tbody>
</table>

**Figure 1. Experimental timeline.** N=50 juvenile female rats, following acclimatization, underwent a wheel-running pre-test. Animals were then randomly assigned to one of 5 treatment groups detailed in Table 1. After 5 full days of experimental conditions, animals were euthanized.
Flibanserin

This study examined the effects of the drug treatment FLIB on the ABA paradigm. Specifically, rats in the ABA-FLIB group were gavaged with 1 ml/kg of FLIB in a saline based solution with 0.5% Natrosol (hydroxyethylcellulose) and 1% polysorbate 80, administered daily (for a period of 5 days) prior to the onset of the dark cycle. A daily dosage of 15mg/kg was chosen based on previous research where FLIB was administered orally by gavage (Ferger et al, 2010; Gelez et al, 2013). Volumes were prepared upon administration based on weight, to ensure the actual dose of the drug received was as close as possible to 15 mg/kg/day. Rats in all other groups were gavaged with a saline vehicle solution (1 ml/kg of 0.5% Natrosol (hydroxyethylcellulose) and 1% polysorbate 80) to eliminate possible procedural effects.

Statistical Analyses

All data will be analyzed using IBM SPSS statistics data editor (version 28). A one-way analysis of variance (ANOVA) was conducted on the initial baseline weight with group (5 levels) as the independent variable and weight (g) as the dependent variable to verify that there were no initial group differences after random assignment.

Since we were interested in changes over time, we conducted mixed-factorial ANOVAs with treatment group (between subjects) and day (within subjects) as independent variables. Dependent variables included weight (g), food intake (g) and running wheel activity per day. Statistically significant interactions were reported for treatment group by time. Tukey t-tests were then conducted for each day and probability values were considered statistically significant if $p < 0.05$. 
Since we were interested in the overall change from the start to end of the experimental manipulations, an overall change was calculated for each measure using the same data. Specifically, weight change (Day 5 - Day 1), change in food intake (Day 5 - Day 1), and change in running activity (Day 5 - Day 1). ANOVA was conducted for each change, with group as the independent variable. Tukey’s HSD post hoc to test multiple comparisons was then done. Probability values were considered statistically significant if p<0.05, after familywise error rate correction.
Results

Activity-Based Anorexia Successfully Modeled at Carleton University

Five rats underwent ABA in our pilot study, and weight matched animals were randomly assigned to 3 control groups: FR (food restriction, no wheel), WH (ad lib food, wheel) and CTRL (ad lib food, no wheel). Weight, food intake and running activity was monitored. We had initially chosen an endpoint of 75% body weight, and 2 of the 5 rats reached this endpoint within 1 week. The study limits were extended as the 3 remaining rats’ weights plateaued within 1 week. By day 14 of the ABA protocol, the remaining 3 rats had not yet reached 75% starting body weight, however they appeared emaciated, had disheveled fur, and one of the 3 showed hypothermia. Given that the rats used in the study were young and still growing, an “adjusted removal criterion” to account for age-related growth was implemented. Although there are no papers describing a removal criterion that accounted for growth, 75% predicted body weight (based on control group weights) was calculated. Based on this calculation, all 5 ABA rats reached 75% of their predicted body weight by day 7 of the protocol. Using these data, the following FLIB protocol’s endpoints were therefore adjusted to either: a specific removal day (day 5) or when body weight fell to 75% predicted weight, whichever occurred earliest.

Flibanserin limits weight loss in ABA

All animals were weighed daily. There were no significant differences observed in baseline weight between the 5 treatment groups ($F_{(4,37)} = 1.87, \ p > 0.05$). We then looked at differences between experimental groups across time. Not surprisingly, an
omnibus ANOVA revealed a significant interaction between experimental group and day \((F_{(16,148)} = 66.837, p < 0.001; \eta^2_p = 0.89)\) (Figure 2). Weight was significantly different between groups on each day except for baseline weight recorded on day 0 (Day 1: \(F_{(2,21)} = 11.93, p < 0.001\); Day 2: \(F_{(2,21)} = 23.23, p < 0.001\); Day 3: \(F_{(2,21)} = 37.68, p < 0.001\); Day 4: \(F_{(2,21)} = 54.39, p < 0.001\); Day 5: \(F_{(2,21)} = 75.49, p < 0.001\) (Figure 2).

**Figure 2. Average weight in groups over time.** Error bars represent daily means ± standard error of the mean (SEM).
Next, to focus specifically on the effect of FLIB on weight, we ran a mixed factorial ANOVA looking at weight over time with the analysis restricted to the ABA-FLIB and ABA-V groups. A significant interaction between group and day was found ($F_{13, 37} = 4.85$, $p = 0.022$, $\eta^2_p = 0.033$) and thus the rate at which weight changes over time depends on FLIB treatment (Figure 3).

![Figure 3](image)

**Figure 3.** Effect of FLIB on weight in ABA. Error bars represent daily means ± SEM.
Finally, we looked at change in weight between day 5 and day 1, and found a significant difference between groups ($F_{(4,37)} = 91.308, p < 0.001; \eta^2_p = 0.91$). Weight change did not differ between WH and CTRL groups (Tukey’s HSD, $p = 0.805$). Not surprisingly, weight change for rats in the CTRL and WH groups were each statistically different from FR, ABA and ABA-FLIB groups. (Tukey’s HSD, $p < 0.001$) (Figure 4), as food restriction results in weight loss. ABA-V rats weight change was significantly different from all groups (Tukey’s HSD, $p < 0.001$), while ABA-FLIB rats weight change was not significantly different from that of FR rats. (Tukey’s HSD, $p = 0.862$) (Figure 4).

**Figure 4. Change in weight between day 1 and 5.** Error bars represent group means ± SEM, data points reflect individual scores. Asterisk (*) denotes significant difference from all other groups ($p < 0.001$). Pound (#) denotes significant difference between ad libitum fed groups and food restricted groups ($p < 0.001$).
Flibanserin increases food intake in ABA

Food intake was measured once daily to observe feeding changes between groups across the study. When we looked at ABA model progression, a significant interaction was found in food intake between groups across time ($F_{(16,148)} = 3.953$, $p < 0.001$; $\eta^2_p = 0.30$) (Figure 5). Food intake was significantly different between groups on each day (Day 1: $F_{(2,21)} = 230.62$, $p < 0.001$; Day 2: $F_{(2,21)} = 280.68$, $p < 0.001$; Day 3: $F_{(2,21)} = 158.11$, $p < 0.001$; Day 4: $F_{(2,21)} = 147.39$, $p < 0.001$; Day 5: $F_{(2,21)} = 116.15$, $p < 0.001$) (Figure 5).

![Figure 5. Average food intake over time. Error bars represent group means ± SEM.](image-url)
The change in food intake between day 5 and day 1 was then calculated to examine the endpoint (Day 5) compared to the start (Day 1), and a difference was observed between groups ($F_{(4,37)} = 7.964, p < 0.001; \eta^2_p = 0.46$) (Figure 6). The change in food intake between ABA-V and ABA-FLIB groups was significant (Tukey’s HSD, $p = 0.004$) (Figure 6). There was no difference in net food intake between ABA-FLIB and the FR group (Tukey’s HSD, $p > 0.05$), while ABA-V net food intake was significantly different from the FR group (Tukey’s HSD, $p < 0.05$) (Figure 6).

![Figure 6. Change in food intake between day 1 and 5. Error bars represent group means ± SEM, data points reflect individual scores. Asterisk denotes significant difference from the 2 other food restricted groups ($p < 0.001$). n.s. denotes not significant ($p > 0.05$)](image)
Flibanserin decreases wheel running in ABA

Our experimental paradigm included some groups that had access to a wheel. Time spent running by these rats was recorded daily and was measured in revolutions. When we considered running behaviour across time, a significant interaction was again found across time between running groups ($F_{(8,84)} = 8.699, p < 0.001; \eta^2_p = 0.45$) (Figure 7). Running behaviour was significantly different between groups on each day except for the first (Day 1: $F_{(2,21)} = 0.44, p > 0.05$; Day 2: $F_{(2,21)} = 6.68, p = 0.006$; Day 3: $F_{(2,21)} = 10.16, p = 0.001$; Day 4: $F_{(2,21)} = 11.20, p < 0.001$; Day 5: $F_{(2,21)} = 17.30, p < 0.001$) (Figure 7).

![Figure 7. Average running over time. Error bars represent group means ± SEM.](image-url)
When we looked at the change in running between day 5 and day 1, we found a significant effect ($F_{(2,21)} = 20.293, p < 0.001; \eta^2_p = 0.66$) (Figure 8). There was no difference in running behavior between ABA-FLIB and WH groups (Tukey’s HSD, $p = 0.627$) (Figure 8). The ABA-V rats increased their running from start to end of the ABA paradigm significantly more than both ABA-FLIB and WH groups (Tukey’s HSD, $p < 0.001$) (Figure 8).

![Figure 8. Change in running between day 1 and 5. Error bars represent group means ± SEM, data points reflect individual scores. Asterisk denotes significant difference from the 2 other groups with wheel access ($p < 0.001$). n.s. denotes not significant ($p > 0.05$)](image-url)
ABA rats removed prior to the 5-day endpoint

There were three rats who were removed early from the experiment, and thus were not included in statistical analysis. The weight, food intake and wheel running activity of these rats are represented in Figure 9.

Figure 9. ABA rats who reached endpoints early. Panel A—daily weight of each rat up until their endpoint. Panel B—daily food intake of each rat up until their endpoint. Panel C—daily wheel running activity of each rat up until their endpoint.
Discussion

Anorexia Nervosa is a debilitating and life-threatening psychiatric disease with important limitations in pharmacologic interventions currently available; psychotherapy remains the most effective course of treatment (Simon, Schmidt, & Pilling, 2005; Brockmeyer, Friederich & Schmidt, 2018). An essential step in using pre-clinical models to uncover new treatments for AN is to validate the ABA model in young female subjects to recapitulate the population characteristics observed in AN. The current study employed a rodent model of AN, ABA in juvenile females, to test the therapeutic potential of FLIB and found that it reversed the effects of ABA such that treated rats had decreased wheel activity, increased food intake and consequently, reduced weight loss compared to vehicle treated ABA rats.

The first experiment in this research program was a pilot of ABA, and although we did not compare males and females in this study, ABA progressed more rapidly in juvenile female rats (< 1 week) than in juvenile male rats (2+ weeks; Pinos et al, 2022), consistent with previous studies. The rapid progression of ABA was also apparent in the experiment using FLIB, as 3 animals were removed before the 5-day endpoint. Overall, these experiments help validate the ABA paradigm in a critical target population: ABA rats displayed high activity levels despite reduced food intake and weight loss.

Many studies have tested the efficacy of existing psychiatric drugs to reverse the effects of ABA, some successfully, including chlorpromazine, which reduced activity and weight loss (Routtenberg & Kuznesof, 1967; Woods & Routtenberg, 1971; Hillebrand et al., 2005), and some with little or no effect on ABA survival, including fluoxetine
(Klenotich et al, 2012). Several experiments used olanzapine in ABA with mixed results (Klenotich et al, 2012; Klenotich et al, 2015). Our study is the first to use FLIB in ABA, and our findings indicate that it is effective in reducing running activity and limiting weight loss, two key symptoms of ABA.

With respect to weight loss, we found that vehicle treated ABA rats were significantly different from all other groups. While the FLIB treated ABA rats still lost weight, their weight loss was not different from the food-restricted control group. Flibanserin treatment, therefore, attenuated weight loss so it was comparable to that of food-restricted controls. Although no animal studies have reported weight gain or loss in animals treated with FLIB, the human HSDD clinical trial data revealed weight loss in women who received FLIB versus placebo (Simon et al, 2019). The researchers hypothesized that the weight loss was due to improvement in mood and sleep, and not due to nausea or increased physical activity (Simon et al, 2019). This suggests that FLIB may increase wellbeing and quality of life, which indirectly resulted in weight loss.

In our analysis of running activity between the 3 groups that had wheel access, we found that the vehicle treated ABA rats ran significantly more than wheel-only and FLIB treated ABA rats. We found no differences in wheel running between the wheel-only and FLIB treated ABA groups, therefore, much like the weight loss analysis, FLIB treatment limited excessive wheel running so that it was comparable to that of a control animal with wheel access. These data provide compelling evidence that FLIB reduces running and weight loss in juvenile female ABA rats. There are a few studies that looked at FLIB and motor behavior. One study found spontaneous motor inhibition in rats treated with 16 mg/kg FLIB via intraperitoneal injection (Borsini et al, 2002). A dose of 32 mg/kg
of FLIB, however, did not reduce motor activity of rat escape behavior in the learned helplessness paradigm or effect rat performance in the Morris maze (Borsini et al, 1999; Borsini et al, 2002). Flibanserin was also found to increase motor activity in rats during amphetamine withdrawal (Borsini et al, 2002), and thus, the actions of FLIB on motor activity and its interactions with different experimental conditions are complex and should be explored in future studies.

When we looked at food intake, we found that the change in food intake between the start and end of experimental conditions was significantly different between vehicle and FLIB treated ABA groups. The change in food intake between FLIB treated ABA animals and animals who were only food restricted was comparable. The difference in net change in the ABA groups is due to a marked decrease in food intake recorded on day 5 in the vehicle treated ABA group. This single day difference in food intake on day 5 between the 3 food restricted groups is interesting and may have been a turning point for vulnerable versus resilient animals: there appears to be a bimodal distribution in the change in food intake in the vehicle treated ABA group. This bimodal distribution may have been stronger if animals who failed to eat in a feeding period were included in calculations, and if no animals were excluded based on running propensity.

The significant difference in food intake and running changes between FLIB and vehicle treated ABA groups suggests that FLIB treatment had a therapeutic effect: it is possible that FLIB treatment increased DA release in the PFC during the feeding period, directing motivation and attention toward food and away from the wheel. The central mechanisms by which FLIB modulates behaviour in the current ABA model are unknown and complicated by the multi-modal actions of the drug. Generally, FLIB has been shown
to decrease 5-HT release and increase DA and NE release in the PFC, whereas inhibition of DA and NE neurons are seen in the brainstem (Baid & Agarwal, 2018). However, FLIB acts as both a 5-HT agonist and antagonist and targets different 5-HT receptor subtypes depending on which brain region is examined (Stahl, 2015). In the PFC, it acts as an agonist at postsynaptic 5HT$_{1A}$ receptors and an antagonist at postsynaptic 5HT$_{2A}$ receptors and antagonist or very weak partial agonist of the D4 receptor (Stahl et al, 2010; Stahl, 2015). This is particularly interesting because studies have shown dysregulation in 5HT$_{1A}$ and 5HT$_{2A}$ receptors in the PFC of patients with AN (Bailer et al, 2004; Bailer et al, 2005). Therefore, it is possible that the specific actions of FLIB on 5HT$_{1A}$ and 5HT$_{2A}$ receptors in the PFC may be involved in its therapeutic effects observed in the current study.

Importantly, DA has been implicated in both AN and the ABA model, such that antagonism of DA receptors has been associated with increased food intake and body weight. However, most of these studies have focused on DA receptors 1-3 and not D4 (Gilbert & Cooper, 1985; Frank et al, 2017) which is the major DA receptor bound by FLIB (Invernizzi et al, 2003; Stahl et al, 2010), therefore the potential for DA’s involvement in the current effects of FLIB are unknown. If FLIB is prescribed to help boost the motivation and reward from sexual activity, it may help with other motivated rewarding behaviors like eating. This might have helped increase food intake in FLIB treated ABA animals.

The work that comprised this research thesis had the objective of determining whether FLIB would be effective in reducing ABA symptoms in juvenile females, to help further AN drug treatment research. We have demonstrated that a low-moderate dose of
FLIB given 1 hour prior to the feeding period throughout the ABA paradigm was sufficient to reduce running, limit weight loss, and induce a positive net change in food intake. As many past animal studies have been done exclusively in males, our research is important overall as it adds a study done in females to the literature. Since AN affects females more frequently than males, using female animals in ABA research is essential; we have shown that the ABA paradigm is valid in juvenile females, and future ABA research should be done in females given the target population in humans. Our findings mark a novel role for FLIB in the treatment of ABA and support the growing evidence of the role of 5-HT modulation in ABA vulnerability and will help direct future research AN etiology. Since FLIB is already approved for use in humans, next steps may include testing in a human eating disorder population.

Limitations

Although the ABA paradigm used in the current study models several key clinical features of restrictive-type AN in humans, such as weight loss and hyperactivity, it is inherently compromised. First, though hyperactivity is common in AN, it is not necessary for diagnostic criteria or present in all patients. Therefore, the ABA model is limited to only a hyperactive sub-phenotype of AN. Second, while the etiology of AN remains unknown, it is likely due to a combination of various environmental and genetic factors that may not be present in the animals exposed to the paradigm. A major confound in AN research is the inability to identify at-risk behaviours or pre-existing conditions prior to the appearance of eating disorder symptomology. Furthermore, body dysmorphia, as well as the pathological fear of becoming “fat” are quintessential psychiatric features of AN
that cannot be modeled in animals. Indeed, AN is a complex human disorder with influenced by pop culture that is not reflected in ABA. It is impossible to model the cultural and cognitive aspects of anorexia nervosa in animals, as no nonhuman animal would relinquish survival and approach death to change their self-concept based on another animal-imposed intention. As such, though informative and imploring further study, important limitations exist in the translatability of these findings.

The current study is limited in that FLIB was only administered to the ABA group, and at a fixed dose. Although it is important to know the effects of FLIB in all other control groups, as well as a dose response, the results of the current study still provide clinical relevancy.

Future studies that examine brain tissue following ABA and FLIB will be necessary to delineate the neurobiological mechanisms that underlie FLIB’s apparent therapeutic action in the ABA model. By co-labelling tyrosine hydroxylase with c-Fos expression, one could examine how FLIB affects DA and NE neurons in the VTA and NAc. Further characterization of the reward circuit in juvenile female ABA rodents would help build on the hypodopaminergic / reward hypothesis of AN. Behavioral measures of anxiety and anhedonia would have been helpful to see if FLIB decreased anhedonia and anxiety commonly seen in ABA and AN and would have allowed for comparison to previous FLIB studies looking at anxiety and depression.

The important medical consequences and stigma associated with EDs in conjunction with the limited and low success rates of AN treatment strategies presents a compelling need to elucidate ED genesis. The current study provides behavioural data that implicate targeting both the dopaminergic and serotonergic pathways as a novel
therapeutic treatment of ABA. Future studies should examine what specific actions of the drug are necessary and sufficient to produce the observed resilience in the ABA model.
References


