**Chapter 1: Introduction**

**1.1 The Brain and Food**

The brain’s relationship with food is changing. As the global food system becomes increasingly saturated with processed and ultra-processed foods, the obesity epidemic grows in parallel (Moubarac et al., 2014). More than half of adult Canadians are overweight or obese and more than 54% of Canadians eat out at least once a week (Moghimi and Wiktorowicz, 2019). Access to these processed foods is easier than ever, and it takes very little energy to get them (Dallman et al., 2006). Ultra-processed foods are more energy dense, with more sugar, sodium, and saturated fats. They are also highly palatable. The typical Canadian’s grocery list becomes increasingly dominated by ultra-processed foods, while unprocessed foods (ingredients) disappear (Moubarac et al., 2014).

Stress, the perceived threat to homeostasis, is an adaptive mechanism that has become a challenge in modern life (Smith and Azevedo, 2025). The body has many mechanisms to return to that homeostasis, vital to resistance and adaption (Smith and Azevedo, 2025). However, these systems have not adapted to our world of high chronic social stress and landscape of easily accessible high calorie, highly palatable foods (Dallman et al., 2006). Chronic stress has been linked to consumption of palatable foods and well as unhealthy eating habits, but the neurophysiological basis for stress eating is unclear (Tryon et al., 2013).

**1.2 Stress**

Physiologically, stress is a challenge to the homeostasis of an organism (Bose et al., 2009), or the perceived threat to homeostasis (Charmandari et al., 2005). When under stress, the organism responds to regain equilibrium (Bose et al., 2009). In humans, stress activates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic adrenomedullary system (SAM) which originates in the locus ceruleus (LC) of the brain stem (Adam and Epel, 2007; Charmandari et al., 2005). In an acute, short-term stressor, the SAM allows for the release of catecholamines which triggers the “fight or flight” response from the sympathetic division of the autonomic system, leading to activation of the HPA axis (James et al., 2023). As part of the HPA axis, paraventricular nucleus (PVN) of the hypothalamus releases corticotrophin releasing hormone (CRH), which stimulates adrenocorticotrophin hormone (ACTH) from the pituitary gland, which in turn stimulates cortisol release from the adrenal cortex (Bose et al., 2009; Adam and Epel, 2007). Under acute stress, cortisol should negatively feedback on CRH and ACTH to prevent prolonged secretion of cortisol (Adam and Epel, 2007; Charmandari et al., 2005). By binding to glucocorticoid receptors, cortisol initiates of represses transcription to negatively feedback on the HPA axis, while binding to mineral corticoid receptors regulates basal HPA activity (Nieuwenhuizen and Rutters, 2008). This tight regulation of cortisol is critical because the acute stress response is necessary for homeostatic recovery, but chronic or prolonged stress can be harmful (Bose et al., 2009).

Chronic elevation of glucocorticoids in the absence of chronic stress inhibits basal HPA activity and HPA activity stimulated by an acute stressor, but this is likely due to inhibition at the pituitary, not central inhibition (Dallman et al., 2006). Norepinephrine neurons in the LC likely activate the HPA, evident by lesioning studies that find a decreases HPA response to acute stress (Dallman et al., 2006). Prolonged activation of the HPA axis suppresses the release of growth hormone, and glucocorticoids, as the final effectors of the HPA, induce insulin resistance (Charmandari et al., 2005). Maybe sentence about social chronic stress here but it would be repetitive to say glucocorticoids are the best marker of the HPA axis responding to psychosocial stress.

**1.2.1 Stress and Appetite**

As part of the normal response to an acute stressor in a stressor-naïve animal, there is afferent activation of the HPA, with glucocorticoid levels increasing within 2-5 minutes of the stimulus (Dallman et al., 2006). This rapid action is critical to shorten the duration of ACTH secretion and the HPA so that the threat can be responded too, but not so much that there could be negative consequences (Dallman et al., 2006). Cortisol, a glucocorticoid, stimulates hunger and feeding (Adam and Epel, 2007). Chronic stress and excess glucocorticoids play a role in obesity by interfering with energy homeostasis (Tamashiro et al., 2011) and increasing food intake and visceral fat deposition (Adam and Epel, 2007).

In the periphery, glucocorticoids act catabolically to mobilize energy stores, ensuring enough fuel for tissues such as the heart and muscles, allowing for the energy to escape stressors (Dallman et al., 2006). In contrast, glucocorticoids act anabolically in the brain, driving caloric intake (Dallman et al., 2006). Meaning of that. This balance and crosstalk between the HPA and SAM are critical in the stress response yet is incredibly complex. SAM produces epinephrine which supresses hunger and digestion (James et al., 2023; Adam and Epel, 2007) but also activates the HPA which produces cortisol which increases hunger.

Under prolonged stress, the ability of glucocorticoids to negatively feedback stimulated ACTH secretion is decreased (Dallman et al., 2003). Chronic stress increases consumption of highly palatable “comfort foods”, reinforcing neural pathways leading to consumption of these foods (Tryon et al., 2013). Factors that predict eating more under stress in humans include being female, overweight, or having a history of food restriction (Adam and Epel, 2007) and women who report more chronic stress also report being emotional eaters (Tryon et al., 2013).

Davies et al. (2023) found females were at higher risk for pandemic stress-induced binge eating, and females ages 10 to 19 showed the greatest increase in eating disorder released hospitalizations during this time (Auger et al., 2023). Hunger and satiety signals are driven by the hypothalamus (Tryon et al., 2013; Smith and Azevedo, 2025). When under stress, this communication is dysregulated, causing alterations in hypothalamic neurons that change feeding behaviours (Smith and Azevedo, 2025).

**1.3 The Dorsomedial Hypothalamus**

The hypothalamus is a tiny, yet powerful brain region that exerts immense control over basic life functions and homeostasis such as energy metabolism and expenditure, autonomic activity, and hormone secretion (Saper and Lowell, 2014; Benedini, 2009; Crosby and Bains, 2012). It is the regulator of the master, pituitary gland and the autonomic nervous system (Benedini, 2009). The hypothalamus integrates multiple signals such as hormonal, metabolic, and neural input both from within the hypothalamus and from other brain regions (Goel et al., 2025; Smith and Azevedo, 2025). Hypothalamic lesioning studies resulted in a wide range of eating behaviours, leading to the distinction and study of distinct hypothalamic nuclei (Anand and Brobeck, 1951).

The dorsomedial hypothalamus (DMH) is a hypothalamic nucleus located adjacent to the third ventricle, dorsal to the ventromedial hypothalamus (VMH), and caudal to the PVN (Paxinos and Watson, 2009). The DMH is involved in energy expenditure, cardiovascular changes in response to stress, thermoregulation, food intake, and body weight regulation (Goel et al., 2025; DiMicco et al., 2002; Tran et al., 2022). The DMH contains a heterogenous population of cells that communicate with various brain regions through both glutamate, the major excitatory neurotransmitter, and GABA (*gamma*-aminobutyric acid), the major inhibitory neurotransmitter (Myers et al., 2014). The DMH receives input from many brain regions including the prefrontal cortex, amygdala, lateral septum, and pre-optic area, while it projects to the PVN, rostral raphe pallidus of the medulla oblongata, and the LC (Myers et al., 2014; Tran et al., 2022).

The DMH is a region of interest due to its integration of satiety and stress signals (Crosby et al., 2011), and an ideal region to study the link between stress and appetite for its role in the regulation of appetite and body weight (Bellinger and Bernardis, 2002), and the presence of receptors that allow these neurons to respond to stress hormones (Myers et al., 2014).

**1.3.1 The DMH and Food Intake**

Early DMH studies in sheep showed that stimulation resulted in hyperphagia, indicating the role of the DMH in appetite (Bellinger and Bernardis, 2002). Lesioning studies in rats later revealed that destruction of the DMH resulted in hypophagia and hypodipsia while maintaining normal body fat percentage and lean body mass (Bellinger and Bernardis, 2002; Dalton et al., 1981). The body weight of adult DMH lesion (DMHL) rats increased at same rate as the controls but never overcame the initial drop after surgery (Dalton et al., 1981).

On a normal diet, young DMHL rats display hypophagia and loose body weight, and in contrast, DMHL rats with restricted diet show immediate hyperphagia. When given high fat diets, DMHL rats become obese compared to regular diet DMHL rats, but not as obese as control animals (Bellinger and Bernardis, 2002). However, on other highly palatable diets, DMHL rats show that can become as obese or even more obese than control animals.

\*Where to put\* Weight loss is a marker of stress in rodents but when they have highly palatable food, stress increases the intake of that highly palatable food (Adam and Epel, 2007).

**1.3.2 The DMH and Stress**

In addition to the role of the DMH in food intake, it plays a major role in the response to and regulation of stress. Glutamatertgic neurons, located primarily in the dorsomedial region of the DMH, and GABAergic neurons, located primarily in the ventrolateral region of the DMH, project to the PVN, respectively activating or inhibiting CRH neurons (Myers et al., 2014). As the DMH is an upstream regulator of the PVN, these projections are modulated by stress. This is evident in stimulation of the dorsal region of the DMH, which results in increased ACTH secretion, while inhibition of this region decreases secretion (Myers et al., 2014).

In addition, activation or disinhibition of DMH neurons is required for the sympathetic response to stress (DiMicco et al., 2002; Crosby and Bains, 2012). When the DMH is stimulated, there is an increase in heart rate, blood pressure, and respiratory rate (Crosby and Bains, 2012). Blocking GABAA receptors produces an increase in heart rate, sympathetic activity, and plasma catecholamines.

Many neurons in the DMH expresses glucocorticoid receptors (Cintra et al., 1990), so the glucocorticoids produced by the HPA may in turn modulate the activity of the DMH, a modulator of the HPA axis. The changes in neuronal activity of DMH neurons in a response to glucocorticoids are unknown but may be playing a major role in the changes in appetite seen under various stressful conditions.

**1.4 Synapses**

Neurons communicate using electrical and chemical (neurotransmitter) signals. Chemical neurotransmission allows for greater flexibility and regulation that electrical transmission and can vary in intensity and speed (Fon and Edwards, 2001). Neurotransmitter is released from a presynaptic neuron and diffuses across the synaptic cleft where the neurotransmitter can bind to receptors on the postsynaptic neuron. The receptors on the postsynaptic neuron determine the nature of the signal (Purves et al., 2001; Fon and Edwards, 2001). The two main families of receptors are ionotropic and metabotropic. Ionotropic receptors are ligand-gated ion channels which change confirmation after binding of a ligand, resulting in an influx or efflux of ions based on the electrochemical gradient. This occurs rapidly, only milliseconds after an action potential reaches the presynaptic terminal and lasts only tens of milliseconds (Purves et al., 2001). Metabotropic receptors, also called G-protein-coupled receptors, undergo a confirmational change when a ligand binds to their extracellular domain that results in various signal transduction cascades, that may directly or indirectly interact with ion channels (Purves et al., 2001). A neurotransmitter may activate both ionotropic and metabotropic receptors at the same synapse, with the complementarity determining the response.

**1.4.1 Glutamate (1.4.1)**

Glutamate, the primary excitatory neurotransmitter in the brain, is produced by the conversion of glutamine by the enzyme glutaminase, which is regulated by phosphorylation (Fon and Edwards, 2001).

Glutamate activates both inotropic and metabotropic receptors (Meldrum, 2000; Siegelbaum and Kandel, 1991). Glutamate acts through three families of ionotropic receptors: N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainite (Meldrum, 2000). AMPA receptor ion channels are permeable to Na+, K+, and sometimes Ca2+, depending on subunit composition, specifically the GluR2 subunit. AMPA have a lower affinity for glutamate than NMDA receptors, but faster kinetics involved in excitatory post synaptic currents (Meldrum, 2000). NMDA receptors are blocked by Mg2+, this blockage is only overcome by partial depolarization, which then allows for the passage of Na+, K+, and Ca2+ (Siegelbaum and Kandel, 1991; Meldrum, 2000). Additionally, glutamate binds to a family of metabotropic receptors containing three groups (mGluR I – III) with specialized functions, which often utilize second messengers like diacylglycerol and cAMP (Meldrum, 2000).

Glutamate is taken up by VGLUT1 and VGLUT2 transporters into vesicles for release into the synaptic cleft (Hertz, 2006). Glutamate in the synapse is taken up by glial cells by GLAST and GLT1, converted to glutamine by astrocyte-specific enzyme glutamine synthase, then back up by neurons via EAAC1 for conversion back to glutamate (Fon and Edwards, 2001; Hertz, 2006), or converted to alpha-ketoglutarate by oxidative deamination or transamination (Hertz, 2006). Something to sort of finish it off.

**1.4.2 Stress and Synaptic Transmission (1.4.2)**

During an acute stressor, a CRH mediated decrease in NMDAR-dependent Ca2+ entry prevents the release of retrograde messengers that normally would block the release of glutamate (Bains et al., 2015), to allow for the stress response. Acute stress elevates extracellular glutamate, dependent on adrenal glands (McEwen, 2017), and likely the HPA axis. When it is time for the acute stress response to come to an end, there is glucocorticoid feedback on the HPA axis to supress glutamatergic synapses to CRH neurons (Levy and Tasker, 2012). The negative feedback from glucocorticoids is dependent on endocannabinoids (eCB), retrograde messengers synthesized as needed in the postsynaptic neuron in response to an increase in Ca2+ or activation of mGluRs (Crosby and Bains, 2012). The eCB 2-arachidonoylglycerol (2-AG) binds to cannabinoid type 1 receptors (CB1R) in the brain, where it supresses adenylate cyclase activity and voltage cation channels, supressing neurotransmitter release (Crosby and Bains, 2012). Under prolonged exposure to cortisol, CB1R expression is reduced, there is less binding of 2-AG, no negative feedback, and the stress response remains activated.

Synaptic plasticity is the alternation of synaptic transmission, either to strengthen or weaken a synapse. Long-term potentiation (LTP) is the prolonged excitation of synaptic transmission, and long-term depression (LTD) the prolonged inhibition (Siegelbaum and Kandel, 1991). LTD does not require GABA-mediated inhibition, as it is enhanced by picrotoxin, a GABAA receptor antagonist. How does LTD/LTP occur?

**1.5 Neuronal Excitability**

At rest, neurons maintain a resting membrane potential, which is the polarization of charge between the intracellular and extracellular environment. Neuronal excitability is characterized by a neuron’s ability to fire an action potential, and a more excitable neuron can fire an action potential at a more polarized resting membrane potential. When glutamate binds to its ionotropic receptors, cations enter the neuron, depolarizing the neuron by making it more positively charged. As the neuron is then more positive, it is closer to the action potential voltage threshold and a smaller change in membrane potential is needed to fire an action potential, demonstrating increased excitability.

**1.5.1 Stress and Neuronal Excitability**

Introduction sentence. Chronic unpredictable stress increases intrinsic excitability of neurons in the hypothalamus in male and female rats (Fang et al., 2023). This may reflect glucocorticoid mediated changes in postsynaptic excitability, as is seen in PVN neurons due to modulation of potassium channels (dos-Santos et al., 2023). As glucocorticoids and HPA axis hormones are modulated by input from the DMH, stress related changes in DMH projections to the PVN could further influence these changes.

**1.6 Current Study**

There is no doubt that the relationship between stress and appetite is heavily intertwined, although the underlying neurophysiological mechanism are unclear. In addition to the role of the DMH in the regulation of food intake (Bellinger and Bernardis, 2002; Dalton et al., 1981) there is considerable evidence for the role of the DMH in autonomic responses seen as a response to stress and presence of stress hormone receptors (Myers et al., 2014; Crosby and Bains, 2012). Stress hormones may be binding in the DMH, altering the neuronal activity and excitability. Since the DMH has a role in both the regulation of food intake and stress response it is an ideal region to study this relationship. The effect of stress on the neuronal transmission and excitability of DMH neurons is unknown but could allow for greater insight/understanding of the sex-based differences in food intake under stress.

The current study aims to answer the question does acute and repeated stress affect glutamatergic/appetite regulating neurons in the DMH of young female rats, and if so, by what mechanism? We hypothesized that stress would alter neuronal excitability and communication in the DMH. We predicted that acute stress would decrease activity of DMH neurons (lesion = less hungry (when given normal diet)… so less activity (cause lesion means no activity) = less hungry) and that chronic stress will increase activity (resistance to GC = hungrier = more activity) of DMH neurons compared to naïve animals. This hypothesis was tested by performing acute and repeated stressors on the animals and assessing the neuronal activity and communication of DMH neurons using patch clamp electrophysiology.